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Sulfonation and Molecular Action

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The sulfonation of endogenous molecules is a pervasive biological phenomenon that is not always easily understood, and although it is increasingly recognized as a function of fundamental importance, there remain areas in which significant cognizance is still lacking or at most minimal. This is particularly true in the field of endocrinology, in which the sulfoconjugation of hormones is a widespread occurrence that is only partially, if at all, appreciated. In the realm of steroid/sterol sulfoconjugation, the discovery of a novel gene that utilizes an alternative exon 1 to encode for two sulfotransferase isoforms, one of which sulfonates cholesterol and the other pregnenolone, has been an important advance. This is significant because cholesterol sulfate plays a crucial role in physiological systems such as keratinocyte differentiation and development of the skin barrier, and pregnenolone sulfate is now acknowledged as an important neurosteroid. The sulfonation of thyroglobulin and thyroid hormones has been extensively investigated and, although this transformation is better understood, there remain areas of incomplete comprehension. The sulfonation of catecholamines is a prevalent modification that has been extensively studied but, unfortunately, remains poorly understood. The sulfonation of pituitary glycoprotein hormones, especially LH and TSH, does not affect binding to their cognate receptors; however, sulfonation does play an important role in their plasma clearance, which indirectly has a significant effect on biological activity. On the other hand, the sulfonation of distinct neuroendocrine peptides does have a profound influence on receptor binding and, thus, a direct effect on biological activity. The sulfonation of specific extracellular structures plays an essential role in the binding and signaling of a large family of extracellular growth factors. In summary, sulfonation is a ubiquitous post-translational modification of hormones and extracellular components that can lead to dramatic structural changes in affected molecules, the biological significance of which is now beginning to be appreciated. (*Endocrine Reviews* 23: 703–732, 2002)

Abbreviations: APS, Adenosine 5′-phosphosulfate; C/EBP, CCAAT/enhancer-binding protein; CCK, cholecystokinin; DHEA, dehydroepiandrosterone; DIT, diiodothyronine; FGF, fibroblast growth factor; FXR, farnesoid X receptor; HCG, human chorionic gonadotropin; IRD, inner-ring deiodination; ORD, outer-ring deiodination; PAP, desulfonated PAPS; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; POMC, proopiomelanocortin; SULT, cytosolic sulfotransferases and their genes; UTR, untranslated region.

I. Introduction

THE BIOTRANSFORMATION OF molecules by sulfonation is a basic metabolic route of primary importance. Sulfonation is the transfer of a sulfonate group (SO_3^{-1}) from the universal sulfonate donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to an appropriate acceptor molecule. Sulfonated compounds comprise a remarkable array of substances, ranging in molecular weight from less than 10^3 to greater than 10^6, that undergo striking changes in their physicochemical properties upon the addition of the highly

charged sulfonate group (1). Sulfonation increases water solubility and can lead to conformational changes in both low- and high-molecular-weight molecules; lipophilic molecules are converted to amphiphiles and, with a pK_a near 1.5, sulfonates remain fully ionized at any pH found in biological systems (1).

Interestingly, two of the more prominent conjugating systems involving the nonmetal elements phosphorous and sulfur sit side by side in the periodic table. In metazoan physiology, phosphorylation and sulfonation are ubiquitous phenomena carried out in all organ systems. Because of the broad role played by phosphorylation in regulatory mechanisms, particularly involving enzymes, signal transduction pathways, and transcription, it continues to receive extensive coverage. The importance of sulfonation, however, is less well appreciated, despite the fact that it is absolutely essential for normal growth and development as well as maintenance of the internal milieu. Sulfonated macromolecules such as glycosaminoglycans and proteoglycans are involved in cell surface and connective tissue structures. The highly acidic and hydrophilic glycosaminoglycans have a major influence on tissue hydration, elasticity, and cation composition (2). Furthermore, they participate directly in high-affinity binding to extracellular matrix proteins, growth factors, enzymes, and cell surface receptors (3–5), and they engage in transmembrane signaling (6). Sulfonation of tyrosine residues is a prevalent posttranslational modification of many secretory and membrane proteins and peptides that may significantly influence functionality (7, 8). Sulfate moieties in sugar residues of glycoprotein hormones have a significant influence on biological activity (9, 10). Sulfoglycolipids such as sphingolipids and galactoglycerolipids are abundant in myelin as well as spermatozoa, kidney, and small intestine (11) and have been implicated in a variety of physiological functions through their interactions with extracellular matrix proteins, cellular adhesive receptors, blood coagulation systems, complement activation systems, and cation transport systems (12). Sulfonation also has a significant role in the biotransformation of many endogenous low-molecular-weight compounds, including catecholamines (13), iodothyronines (14), and vitamin C (15). Likewise, sulfonation is an important modification of cholesterol (16) and its derivatives, bile acids (17), vitamin D (18, 19), and steroids (20).

The principal aim of this review is to elaborate on the mechanism and function of sulfonation in a number of the basic systems noted above, especially those related to endocrinology. The primary intent is to summarize the current status of sulfoconjugation in these systems, particularly as this modification applies to molecular mechanisms operative in human endocrine physiology. One area that will be touched on in the review involves the extracellular matrix and proteoglycans, the latter being a term not exactly in the working lexicon of most endocrinologists. However, sulfonated proteoglycans and their role in cell signaling is a subject of great importance, especially regarding the molecular action of specific growth factors. In the final analysis, it is hoped that the reader will come away from the review with an enhanced appreciation for the pervasiveness as well as importance of sulfonation in mammalian biology.

II. The Universal Sulfonate Donor Molecule

A. General

The elements carbon, nitrogen, and sulfur are made available to organisms chiefly in the form of inorganic compounds. For sulfur, the inorganic compound is sulfate. Furthermore, as in the case of carbon dioxide formation and nitrogen fixation, the utilization of sulfate also requires metabolic activation to a form that can be reduced to sulfide, which is then used in the production of cysteine and methionine needed for the synthesis of protein (21). Multicellular organisms, however, are unable to reduce sulfate to sulfide, just as they cannot reduce carbon dioxide to carbohydrate and nitrate to ammonia. Thus, the animal kingdom must rely on plants and bacteria to provide the reduced forms of these elements (21). In the case of sulfur, the activated sulfate compound is PAPS (22, 23). The importance of PAPS is that it serves not only as a substrate for reduction by bacteria and plants but also as the active agent for sulfonate esterification, a process carried out by all organisms. PAPS thus serves as the universal sulfonate donor molecule required for all sulfonation reactions (24); in mammals, all tissues are able to carry out the synthesis of PAPS (25).

B. PAPS synthesis

PAPS synthesis requires a ready supply of sulfate, which is available from the diet as well as catabolism of proteins and sugar sulfates (1). Abnormalities resulting from sulfate deficiency are theoretically possible but are distinctly unusual under normal circumstances, because plasma levels of sulfate are resistant to manipulation (1). There are, however, genetic disorders involving the cellular uptake of sulfate by a carrier-mediated transport (26). Achondrogenesis type 1B is a recessively inherited chondrodysplasia characterized by extremely poor skeletal development and perinatal death (27). Atelosteogenesis type II is a recessively inherited neonatally lethal chondrodysplasia characterized by defective uptake of inorganic sulfate and insufficient sulfonation of macromolecules (28). These two genetic disorders, and a third recessively inherited but nonlethal chondrodysplasia (diastrophic dysplasia), are caused by mutations in the diastrophic dysplasia sulfate transporter gene located on the long arm of chromosome 5 (29). In the three conditions, impaired sulfate transport across cell membranes results in undersulfonation of cartilaginous proteoglycans. Phenotypic severity correlates with the underlying sulfate transporter gene mutations: homozygosity or compound heterozygosity for stop codons, or transmembrane domain substitutions, usually result in achondrogenesis type 1B, whereas other structural or regulatory mutations result in less severe phenotypes (29). Interestingly, Pendred's syndrome (sporadic goiter with impaired iodine organification and congenital sensorineural deafness) is an autosomal recessive disorder caused by a mutated gene located on chromosome 7q22–31.1. The Pendred syndrome gene, which produces a transcript that is expressed in the thyroid, the inner ear, and the kidney (30, 31), was originally thought to be a sulfate transporter gene (32), because the predicted protein, pendrin, has high homology to several sulfate transporters found in yeast,

plants, and animals (30). Subsequently, however, it was shown that pendrin, which belongs to a large family of anion transporters (32), functions as a transporter of chloride and iodide but not sulfate (33). Nevertheless, it is of interest that one of pendrin's closest mammalian relatives is the protein encoded by the diastrophic dysplasia sulfate transporter gene (34).

The activation of inorganic sulfate to form PAPS results from the concerted action of two enzyme systems (35–37), which in animal species is carried out by a bifunctional protein (38). The first step (Fig. 1) is catalyzed by ATP sulfurylase and involves the reaction of inorganic sulfate with ATP to form adenosine 5′-phosphosulfate (APS) and inorganic phosphate. This reaction results in the formation of a high-energy phosphoric-sulfuric acid anhydride bond that is the chemical basis for sulfate activation (39). The second step (Fig. 1) is catalyzed by APS kinase and involves the reaction of APS with another molecule of ATP to form PAPS and ADP. Unlike ATP sulfurylase, APS kinase is not involved in the activation of sulfate, and its raison d'être is not well understood (39). In an interesting departure from sulfonation reactions, it was found that PAPS could also serve as a phosphate donor in protein phosphorylation. That is, the 3′-phosphate group of PAPS was transferred to a serine residue in an 85-kDa membrane protein (40). Furthermore, this phenomenon was carried out by 11 different tissues examined, suggesting the existence of a novel widespread form of phosphorylation (40). This intriguing *in vitro* finding notwithstanding, its physiological significance remains to be elucidated.

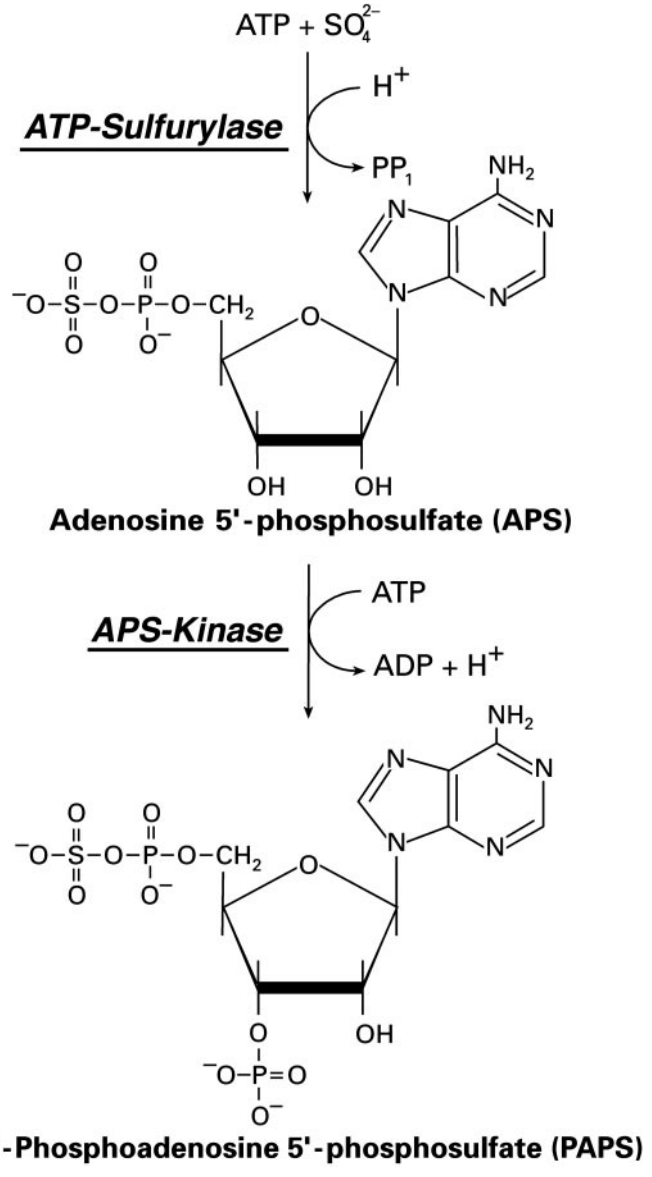

FIG. 1. Catalytic reactions in the formation of PAPS.

In bacteria, fungi, yeast, and plants, ATP sulfurylase and APS kinase are located on separate polypeptide chains. As noted above, however, as a result of gene fusion, the two enzymes are integral to a single protein in animal species (38). The finding that ATP sulfurylase and APS kinase are contained within an individual bifunctional protein (PAPS synthase) led to the discovery that transfer of the intermediate APS from the sulfurylase catalytic center to the kinase active site (*cf.* Fig. 1) involves a channeling process with an efficiency of approximately 96% (41, 42).

C. Cloning and characterization of PAPS synthases

PAPS synthase exists as two isozymes encoded by genes located on separate chromosomes. PAPS synthase 1 has been cloned from several species, including the marine worm (43), mouse (44), human (45–47), *Drosophila* (48), and guinea pig (49). The gene for human PAPS synthase 1 is located on chromosome 4q25–26 (50). Human PAPS synthase 1 is 98% and 95% identical with mouse and guinea pig PAPS synthase 1, respectively, indicating that it is a highly conserved protein. The catalytic domain of APS kinase is located in the amino-terminal region, whereas the ATP sulfurylase domain is in the carboxy-terminal section of this bifunctional protein (45). The division between the two domains, as determined for human PAPS synthase 1, is at the junction of amino acids 226 and 227 of the 624-amino-acid protein (*cf.* Fig. 2; author's unpublished data). Interestingly, there are two completely conserved nucleotide-binding motifs, one in each domain (*cf.* Fig. 2).

As indicated in Fig. 1, during PAPS formation two molecules of ATP are required, one for each reaction. Sequence analysis revealed the presence of a nucleotide-binding P-loop motif (GxxGxGKS/T) in the amino-terminal APS kinase domain of all cloned PAPS synthase proteins (*cf.* Fig. 2). The P-loop motif is highly conserved among nucleotide-binding proteins, in which it is involved in coupling to the phosphate moiety of ATP and cleavage of the β-γ phosphodiester bond (51, 52). Site-selected mutagenesis of the P-loop motif in mouse PAPS synthase 1 markedly impairs APS kinase activity (53), a finding consistent with the hypothesis that the P-loop motif is involved in cleavage of the β-γ phosphodiester bond of ATP with transfer of the terminal phosphoryl group of ATP to the 3′-hydroxyl position of APS (Fig. 1). In contrast to the amino-terminal APS kinase domain, the carboxy-terminal ATP sulfurylase domain of PAPS synthase does not contain a classical P-loop motif for ATP binding. Although the carboxy-terminal ATP sulfurylase domain of

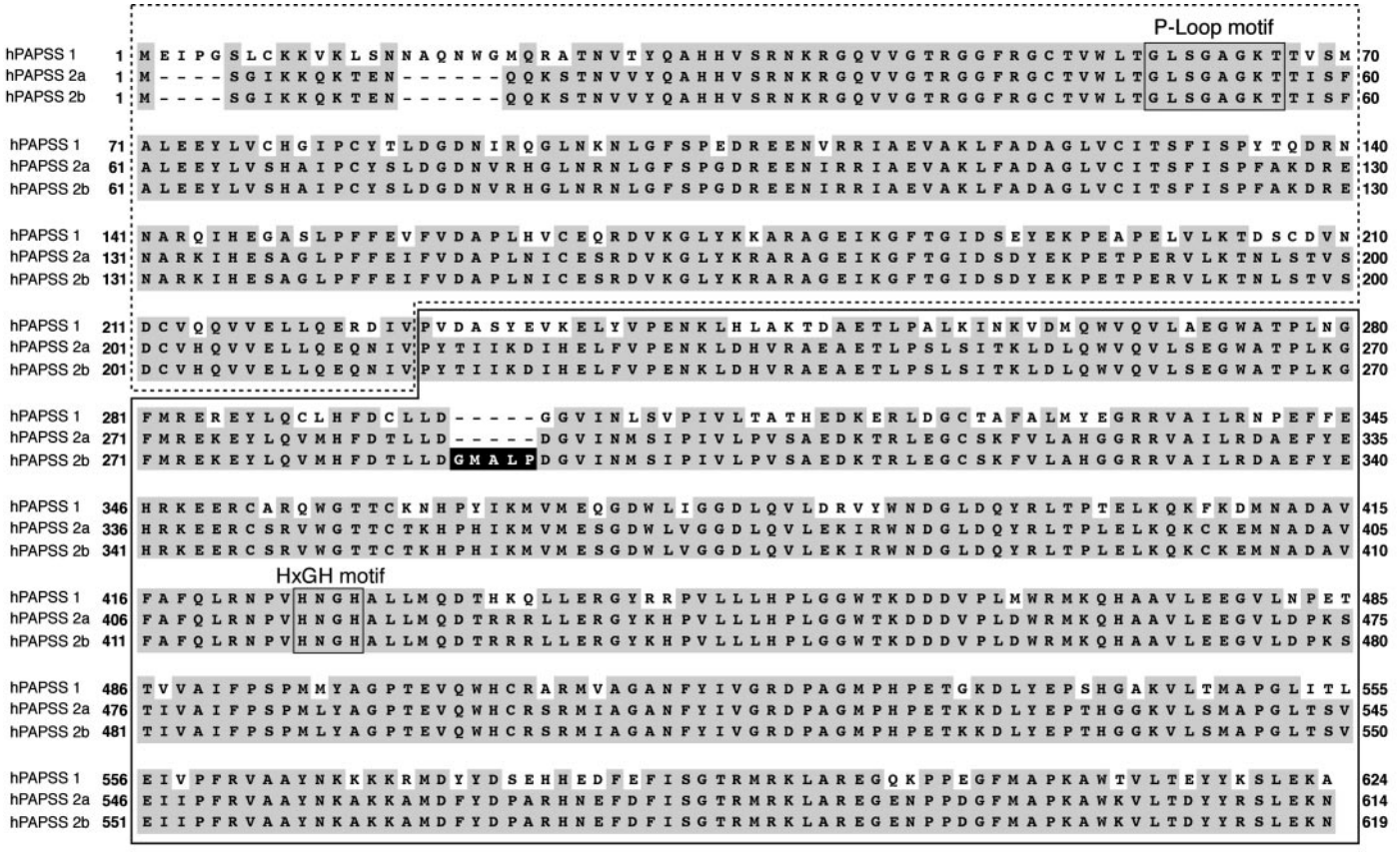

FIG. 2. Amino acid alignment of human PAPS synthase 1 (hPAPSS1), human PAPS synthase 2a (hPAPSS2a), and human PAPS synthase 2b (hPAPSS2b). The *dashed box* defines the APS kinase domain, and the *external solid box* outlines the ATP sulfurylase domain. The P-loop motif in the APS kinase domain and the HxGH motif in the ATP sulfurylase domain are *boxed and labeled*. The GMALP sequence in the ATP sulfurylase domain of hPAPSS2b is shown by *reverse type*. Identities are *shaded*.

PAPS synthase binds ATP, as does the amino-terminal domain, the ultimate fate of the bound ATP differs. Whereas APS kinase catalyzes removal of the terminal γ-phosphate for transfer to the acceptor molecule, APS, ATP sulfurylase catalyzes removal of the β-γ diphosphate (inorganic phosphate) of ATP and condensation of the formed AMP with inorganic sulfate to form APS (*cf.* Fig. 1). The latter function requires a different type of nucleotide-binding site that functions as an α-β phosphodiesterase rather than a β-γ phosphodiesterase. Thus, the carboxy-terminal domain of all cloned PAPS synthase proteins contains another type of nucleotide-binding motif, HxGH (*cf.* Fig. 2). This motif has been characterized as the signature of a large family of nucleotidylyltransferases that cleave the α-β phosphate bond of a nucleotide (54). As with the P-loop motif, mutational analysis of the HxGH motif confirms its role in ATP sulfurylase activity (55).

After the cloning of PAPS synthase 1, a second isozyme (PAPS synthase 2) was cloned for human (56), mouse (56, 57), and guinea pig species (49). The gene for human PAPS synthase 2 has been localized to chromosome 10q23–24 (56). The mouse and human PAPS synthase 2 isozymes were discovered through investigation of specific developmental dwarfing disorders, *i.e.*, brachymorphism in mice and a form of spondyloepimetaphyseal dysplasia in humans. In both human and mouse species, the PAPS synthase 1 and 2 proteins are 77% identical. An interesting feature of the human and mouse PAPS synthase 2 genes is the occurrence of alternative splicing (58), which results in the formation of two variants (2a and 2b) distinguished by the presence or absence of a five-amino-acid segment (GMALP) in the ATP sulfurylase domain of the protein (*cf.* Fig. 2). The catalytic activity of the human PAPS synthase 2a splice variant is modestly but significantly less (~30%) than that of the 2b variant (49).

The physiological significance of the two genes encoding for related proteins that carry out an identical function, *i.e.*, synthesis of the essential sulfonate donor molecule, PAPS, is presently unclear. Interestingly, the catalytic activity of the PAPS synthase 2 variants is 10- to 15-fold higher than that for PAPS synthase 1 (49). It is difficult to imagine that this represents a backup system, because the human PAPS synthase 2 variants appear to be expressed in a tissue-specific manner in contrast to the ubiquitously expressed PAPS synthase 1 (49). What then is the relationship of PAPS synthase 1 to PAPS synthase 2? It was recently reported that PAPS synthase 1 has a nuclear localization in mammalian cells; on the other hand, PAPS synthase 2 has a nuclear localization only when coexpressed with PAPS synthase 1 (59). It was suggested that PAPS synthase 1 localizes to the nucleus in most cells, whereas PAPS synthase 2 localizes to the cytoplasm in tissues in which PAPS synthase 1 levels are low. Interestingly, it was determined that nuclear targeting of PAPS syn-

thase 1 requires the 21-amino-acid segment from the catalytically dispensable amino terminus (59). This intriguing hypothesis notwithstanding, it is difficult to understand the physiological significance. Although some sulfonation may necessarily occur in the nucleus, *e.g.*, estrogen sulfotransferase in the guinea pig and rat has a nuclear localization (60, 61), it would appear that the cytoplasmic compartment is the major site of sulfonation involving secretory proteins, proteoglycans, glycosaminoglycans, galactoglycerolipids, sphingolipids, and a myriad of small-molecular-weight compounds such as hormones and neurotransmitters, as well as drugs and xenobiotics.

Human PAPS synthase 2 was discovered during a search for the genetic basis of a developmental abnormality resulting in a form of spondyloepimetaphyseal dysplasia that presents with a skeletal phenotype involving the spine and long bones. This recessive dwarfing disorder is caused by a nonsense mutation located in the ATP sulfurylase domain of PAPS synthase 2 (56). Brachymorphism, a comparable dwarfing abnormality occurring in mice, is due to a mutation in the syntenic gene for PAPS synthase 2; in this case, there is a missense mutation in the APS kinase domain of the protein (57). It was baffling that in the genetic disorder involving human PAPS synthase 2, which produces the osteochondrodysplasia phenotype, the cartilage-specific defect occurs despite the coexpression of PAPS synthase 1 in cartilaginous tissue (56). In fact, in adult human cartilage, PAPS synthase 1 appears to be the dominant isoform (49). This apparent enigma would seem to be resolved by examination of cartilage from guinea pigs as an animal model. Similar to humans, cartilage from mature animals predominantly expresses PAPS synthase 1. In contrast, however, PAPS synthase 1 expression is relatively low in the cartilage of immature guinea pigs including the growth plate of long bones, whereas PAPS synthase 2 is the vigorously expressed isozyme (49).

Defects involving the PAPS synthase 1 gene have not been reported, presumably because it is ubiquitously expressed, in contrast to PAPS synthase 2; furthermore, it is the predominant, if not sole, isoform expressed in the central nervous system and bone marrow, in which genic mutations are likely to be embryologically lethal.

D. Regulation of PAPS availability

The fact that PAPS is such a strategic biological molecule makes understanding the molecular mechanisms involved in regulating its availability of vital importance. Information in this area, however, is presently rather limited. Little is known, for instance, regarding the transcriptional regulation of the PAPS synthase genes, or the stability of mRNA encoding PAPS synthase proteins, or specific tissue turnover of PAPS synthase proteins. Proximal promoter regions of the genes for human PAPS synthase 1 and 2, which contain neither a TATAAA nor a CCAAT box, have been identified and, in each case, found to be under the influence of the Sp1 family of transcription factors (62, 63). Although these findings are of some interest, they nevertheless represent only incipient observations on the transcriptional regulation of these important genes.

The principal PAPS-degrading enzymes in animal tissues are nucleotidases and sulfohydrolases (23, 64, 65). A 3′-nucleotidase converts PAPS to APS and PAP (desulfonated PAPS) to AMP by hydrolysis of the 3′-phosphate bond (66, 67). A 5′-nucleotidase hydrolyzes the 5′-phosphosulfate bond of PAPS and APS (68–70). A sulfohydrolase converts PAPS to PAP and APS to AMP (71–73). PAPS-metabolizing enzymes appear to be widely distributed and are localized to both particulate and soluble tissue fractions (23, 64, 65). In animal species, none of these enzymes has been suitably purified and adequately characterized, and except for the cloning of a 3′,5′-bisphosphate nucleotidase in yeast, no PAPS-metabolizing enzyme has been cloned (74, 75). Although it can be postulated that such enzymes are involved in the regulation of tissue concentrations of PAPS, there are no pertinent experimental results that address this issue. It has been suggested that high tissue concentrations of APS might inhibit the APS kinase reaction (76). This suggestion is based on a study of the fungus *Penicillium chrysogenum* (77), a species in which ATP sulfurylase and APS kinase are on separate polypeptide chains and channeling of APS from ATP sulfurylase to APS kinase does not occur (78). On the other hand, bifunctional PAPS synthase does demonstrate channeling of APS (42); therefore, it would seem unlikely that exogenous APS would significantly inhibit the APS kinase reaction, a conclusion supported by recent experimental evidence (49).

E. Tissue levels of PAPS

There is a limited amount of information available regarding organ concentrations of PAPS in different species under presumably basal metabolic conditions. For instance, in rat liver and kidney, PAPS levels range from 60–160 and 40–50 nmol/g tissue, respectively (79–83). The concentration of PAPS in lung, intestine, and brain, as well as in liver and kidney tissues of the rat, mouse, hamster, rabbit, and dog, indicates that for each species the highest level is in the liver and ranges from approximately 15 nmol/g tissue for the dog to approximately 80 nmol/g tissue for the rat (84); the concentration of PAPS in various other tissues varies between 6 and 20 nmol/g tissue, with no significant species or sex differences (84). The level of PAPS in several human tissues, including liver, lung, kidney, ileum, and colon, ranges from approximately 4 nmol/g tissue in the lung to approximately 23 nmol/g tissue in the liver (85). The level of PAPS in human fetal liver is approximately 10 nmol/g tissue *vs.* approximately 23 nmol/g tissue in adult liver and approximately 4 nmol/g tissue in the placenta (86).

Tissue (mostly liver) levels of PAPS have been monitored during various manipulations, *e.g.*, during a sulfur-deficient diet, treatment with inhibitors of energy metabolism, and the administration of xenobiotics to accelerate sulfonation. Such studies, performed primarily in rats, demonstrate the ability to experimentally modulate hepatic levels of PAPS. There are, however, no published reports on correlating production and metabolism of PAPS with a sulfonation step for any tissue. Thus, the influence of PAPS availability on a specific sulfoconjugation reaction under strict experimental condi-

tions, involving either endocrine or metabolic manipulations, remains to be investigated.

III. Sulfotransferases

A. General

Sulfonation reactions are usually classified by the acceptor group involved in sulfoconjugation, *e.g.*, *O*-sulfonation (ester), *N*-sulfonation (amide), and *S*-sulfonation (thioester) (1). *O*-Sulfonation involves an alcohol group and can occur with diverse, relatively small endogenous compounds such as catecholamines, steroids, thyroid hormones, and vitamins. Similarly, macromolecules such as glycosaminoglycans, proteoglycans, proteins, and galactoglycerolipids are subject to *O*-sulfonation. In general, *O*-sulfonation represents the dominant cellular sulfonation reaction. *N*-Sulfonation, although relatively less prominent than *O*-sulfonation (25), is nevertheless a crucial reaction in the modification of carbohydrate chains in macromolecules such as heparin and heparan sulfate proteoglycans (87, 88). *N*-Sulfonation is also involved in the metabolism of xenobiotics (89). *S*-Sulfonation is not relevant to this review.

B. Classification

Sulfotransferases can be divided into two classes based upon whether they are soluble or membrane-associated proteins. Soluble or cytosolic sulfotransferases sulfonate a wide variety of endogenous compounds including hormones and neurotransmitters as well as drugs and xenobiotics. Membrane-associated sulfotransferases are located in the *trans*-Golgi complex, where they are involved in the posttranslational modification of macromolecules such as secretory proteins and glycosaminoglycans. There is an effort afoot to establish a standard nomenclature for cytosolic sulfotransferases stemming from an international workshop on sulfation held a few years ago in Drymen, Scotland. The term SULT was adopted as an abbreviation for cytosolic sulfotransferases and their genes (90). Although this is not as yet the "official" nomenclature, it will nevertheless be the general form used in this review.

1. Cytosolic sulfotransferases. This class of sulfotransferases embodies an ever-enlarging superfamily of enzymes that catalyze the sulfonation of relatively small endobiotics and exobiotics. In mammals, at least 44 cytosolic sulfotransferases have been identified that comprise five SULT families that share less than 40% similarity with each other (91). Of these five SULT families, the first two represent the largest and most widely examined families. The SULT1 family consists of sulfotransferases that transfer sulfonate to phenolic drugs and catecholamines (SULT1A; human chromosome 16), estrogenic steroids (SULT1E) and thyroid hormones (SULT1B; human chromosome 4), and xenobiotics (SULT1C; human chromosome 2). The SULT2 family members primarily sulfonate neutral steroids (SULT2A) and sterols (SULT2B; human chromosome 19). Enzymes within the SULT3 family catalyze the formation of sulfamates, whereas the SULT4 and SULT5 families (human chromosome 22) consist of unique cDNAs found in the DNA database and have not yet been adequately characterized (91). Chromosomal clustering of subfamily members suggests that the emergence of isozymes has probably resulted from gene duplication.

Recently, brain-specific sulfotransferases that have less than 36% amino acid sequence identities to other known cytosolic sulfotransferases have been cloned from human (92, 93), rat (92), and mouse (93). These novel, highly conserved (98% identical) cytosolic sulfotransferases, whose endogenous substrate(s) are unknown, appear to belong to a family of cytosolic sulfotransferases distinct from the two major families noted above, and they have been designated SULT4A1 (94).

The biotransformation of endogenous substrates and xenobiotics by sulfonation is a major metabolic reaction that has two possible consequences, *i.e.*, activation or inactivation of a biological effect. An example of the former involving an endobiotic is the conversion of pregnenolone to pregnenolone sulfate, which is a potent neuroexcitatory agent by virtue of its antagonistic action on the γ-aminobutyric acid $(GABA)_A$ receptor that regulates chloride channels (95). Another example in which sulfoconjugation leads to activation, in this case involving a xenobiotic, is the drug minoxidil, a potent vasodilator and trichogen whose active form is minoxidil sulfate (96). On the other hand, inactivation of a biological effect can also be produced by sulfoconjugation. For instance, the genomic action of steroid hormones is inhibited by sulfoconjugation because the sulfates of steroid hormones are unable to bind to their cognate nuclear receptors (97). With a few notable exceptions (such as minoxidil), sulfoconjugation is an important mechanism in the inactivation and excretion of drugs and xenobiotics (1, 25). Nevertheless, whereas xenobiotics are usually detoxified by sulfonation, it is noteworthy that a number of compounds (procarcinogens) are converted into highly reactive intermediates by sulfonation and can then act as chemical carcinogens and mutagens by covalently binding to DNA (98–100). The *O*-sulfonated product of tamoxifen, the drug widely used in the treatment of breast cancer, is a hepatocarcinogen in rats but not in humans (101), although there is a suggestion that tamoxifen can be genotoxic in humans (102).

2. Golgi-associated sulfotransferases. This class of sulfotransferases is primarily concerned with the posttranslational modification of carbohydrates, peptides, and proteins. Carbohydrate sulfotransferases are resident transmembrane enzymes of the Golgi network that recognize sugar residues attached to lipids and proteins passing through the secretory pathway (5). In mammals, 32 different carbohydrate sulfotransferases have been cloned and characterized to date (103). Importantly, carbohydrate sulfotransferases are stereoselective and exhibit strict substrate specificities.

Carbohydrates attached to lipids and proteins display complex and heterogeneous structures, and the addition of a sulfonate group can transform a common structural motif of a carbohydrate into a unique recognition site for a specific receptor or lectin (9). One consequence relates to the control of the circulatory half-life of glycoprotein hormones (10). Extracellular sugars are also frequently sulfonated and as a result can mediate numerous highly specific molecular-recognition events such as the binding of growth factors (87).

For many proteins, tyrosine sulfonation is important for biological activity and correct cellular processing (104). Sulfonation is the most abundant posttranslational modification of tyrosine residues involving many soluble and membrane proteins transiting the secretory path (7). An example of tyrosine sulfonation being essential for biological activity is that of cholecystokinin (CCK), which is 250 times more potent in the sulfonated form than in the unconjugated form (105). Tyrosylprotein sulfotransferases are membrane-bound residents of the *trans*-Golgi network (8), and, to date, two isozymes have been identified and characterized (106, 107).

C. Biochemistry

Sulfonation refers to the transfer of an SO_3^{-1} group, whereas sulfation refers to the transfer of an SO_4^{-2} group; thus, the products of sulfonation should correctly be referred to as sulfonates (1). However, because transfer of an SO_3^{-1} group to a hydroxyl acceptor creates an SO_4 moiety (Fig. 3), sulfonated products have been incorrectly termed "sulfates", a nomenclature that is deeply entrenched. In the general scheme of sulfonation, the sulfonate acceptor (ROH) and donor (PAPS) molecules bind to a sulfotransferase with subsequent release of the sulfonated product and desulfonated PAPS, *i.e.*, PAP (Fig. 3). Sulfotransferases are characteristically high-affinity and low-capacity enzymes. As a consequence, the activity of sulfotransferases is 2–3 orders of magnitude slower than that of phosphotransferases (1). Hydrolysis of the sulfonated product and regeneration of the substrate as a free alcohol complete the sulfurylation cycle, a process carried out by members of the sulfohydrolase or sulfatase gene family (108). Although sulfohydrolysis is extremely important in the overall sulfurylation scheme, this aspect of the sulfurylation cycle will not be covered in this review.

D. Structure

The crystal structures of four cytosolic sulfotransferases have been determined: mouse estrogen sulfotransferase (109), human dopamine/catecholamine sulfotransferase (SULT1A3; Refs. 110 and 111), human hydroxysteroid sulfotransferase (SULT2A1; Ref. 112), and human estrogen sulfotransferase (113). In addition, the crystal structure of the sulfotransferase domain of the bifunctional Golgi enzyme heparan sulfate *N*-deacetylase/*N*-sulfotransferase-1 has been determined (114). Interestingly, specific structural features involved in the binding of the PAPS cofactor are completely conserved in both the cytosolic and Golgi-membrane sulfotransferases, a finding strongly suggesting that both classes of sulfotransferases evolved from a common ancestral gene (115). Elucidation of the sulfotransferase crystal structures notwithstanding, compositional features that determine substrate specificity, despite some recent progress, remain to be clearly established (115).

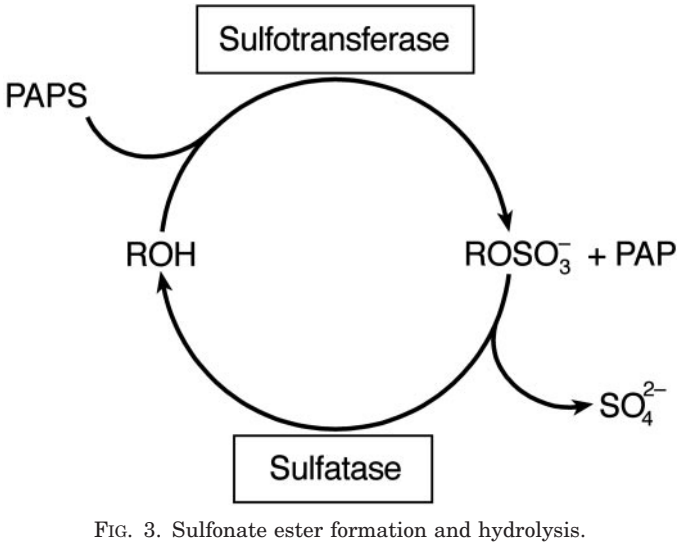

FIG. 3. Sulfonate ester formation and hydrolysis.

IV. Sulfonation of Steroids/Sterols

A. General

The sulfonation of steroids has received considerable attention during the past decade, largely as a result of cDNA cloning of sulfotransferases from a variety of mammalian species. Because this subject was summarized as recently as 5 yr ago (116, 117), this review will primarily focus on significant developments that have occurred during the interim period. An important advance has been the cloning of a unique hydroxysteroid sulfotransferase subfamily with distinctly novel expression and substrate specificity. A substantial payoff of recombinant DNA technology has been the ability to produce large quantities of a protein for characterization studies and crystallization, as well as the ability to produce altered versions of a specific protein for structure/function analyses. The latter has been an active area of investigation in sulfotransferase research with significant progress in establishing specific structural principles. Additionally, there have been further developments regarding a multiplicity of biological roles attributable to steroid sulfates, as well as the enzymes responsible for their generation.

B. Novel steroid/sterol sulfotransferase subfamily

The cloning of a unique hydroxysteroid sulfotransferase subfamily in human (118) and mouse (119) species has significantly moved the steroid sulfotransferase field forward. The novel human hydroxysteroid sulfotransferase gene (*SULT2B1*) was discovered during a search of an expressed sequence tag database using a probe containing a highly conserved sulfotransferase sequence (118). The search yielded an expressed tag located at the 3′-end of a clone isolated from a human placental cDNA library by the I.M.A.G.E. Consortium that led to the cloning of two cDNAs designated SULT2B1a and SULT2B1b. These subtypes, which result from the use of an alternative exon 1, thus encode for proteins that differ only at their amino termini. The *SULT2B1* gene maps to chromosome 19q13.4, approximately 500 kb telomeric to the location of the related *SULT2A1* gene (118).

SULT2A1, the prototypical human hydroxysteroid sulfotransferase (120, 121), is commonly referred to as dehydroepiandrosterone (DHEA) sulfotransferase, because DHEA is

considered the major substrate. SULT2A1, however, has broad substrate specificity and will sulfonate a wide variety of steroids and sterols, in addition to DHEA, involving hydroxyl groups at different carbon locations and with different spatial orientations. A 3α-hydroxyl group (androsterone and bile acids), a 3β-hydroxyl group (DHEA and pregnenolone), a 17β-hydroxyl group (testosterone and estradiol), and a phenolic hydroxyl group (estradiol and estrone) are sulfonated by human SULT2A1 (122–126). Although human SULT2A1 preferentially sulfonates DHEA, it negligibly sulfonates cholesterol, which is the reverse of the case with human SULT2B1 (127). Similar to SULT2A1, the SULT2B1 isoforms will sulfonate pregnenolone; however, in contrast to SULT2A1, they do not sulfonate androsterone, bile acids, testosterone, estrogens, or cortisol (118, 127–129). In more recent detailed studies, the human SULT2B1 isoforms demonstrate striking differences in substrate specificity. That is, SULT2B1a shows a distinct preference for pregnenolone as a substrate, whereas cholesterol is only minimally sulfonated (Fig. 4). On the other hand, SULT2B1b vigorously sulfonates cholesterol, whereas pregnenolone is less avidly metabolized (Fig. 4). Additionally, in contrast to SULT2A1, which also does not sulfonate cholesterol, neither SULT2B1 subtype sulfonates DHEA efficiently (Fig. 4). These results suggest that the human SULT2A1 and SULT2B1 isozymes have selective physiological roles and that the SULT2B1b isoform functions as a true cholesterol sulfotransferase.

Of further interest is the finding of strict structural requirements for SULT2B1b action. For instance, SULT2B1b will sulfonate the 3-hydroxyl group of C_{27} sterols but not other hydroxyl groups such as a hydroxyl group at the C-27 position (127). Furthermore, SULT2B1b demonstrates specific requirements regarding spatial orientation of the 3-hydroxyl group as well as structural conformation of the perhydrocyclopentanophenanthrene ring. That is, a planar arrangement of the fused rings and a β orientation of the 3-hydroxyl group are essential structural features, as illustrated in Fig. 5. The 5α-reduced form of cholesterol (cholestanol), a planar molecule like cholesterol, is approximately 70% as effective a substrate as cholesterol, whereas catalytic efficiency falls to 20–25% with the 5β-reduced form (coprostanol), a nonplanar molecule containing a sever bend. Spatial orientation of the 3-hydroxyl group is also crucial, as shown by the fact that the 3α-hydroxy stereoisomer of cholesterol (epicholesterol) is an extremely poor substrate (Fig. 5).

Although the human SULT2B1 isoforms are considered to be hydroxysteroid sulfotransferases, they are nevertheless structurally unique when compared with SULT2A1 as well as with other cognate mammalian cytosolic sulfotransferases that have been cloned. For example, in comparing the SULT2A1 and SULT2B1 proteins, the outstanding distinction is the extended amino- and carboxy-terminal ends of the latter (*cf.* Fig. 6). Overall, the proteins are approximately 37% identical. However, if the unique amino- and carboxy-terminal ends of the SULT2B1 isoforms are excluded, identities increase to approximately 48%. All previously cloned members of the cytosolic sulfotransferase superfamily, *i.e.*, estrogen and phenol sulfotransferases, as well as the hydroxysteroid sulfotransferases, have sizes that range from 282 to 295 amino acids (130, 131), whereas the human SULT2B1 isoforms consist of 350–365 amino acids (*cf.* Fig. 6). The unique extended amino- and carboxy-terminal ends of the SULT2B1 isoforms notwithstanding, there is a significant structural similarity between the SULT2A1 and SULT2B1 proteins in their core regions. Most notably, a PSB loop (another type of P-loop motif found at phosphate binding sites of nucleotide-binding proteins) and specific amino acid residues important in protein-cofactor interaction of cyto-

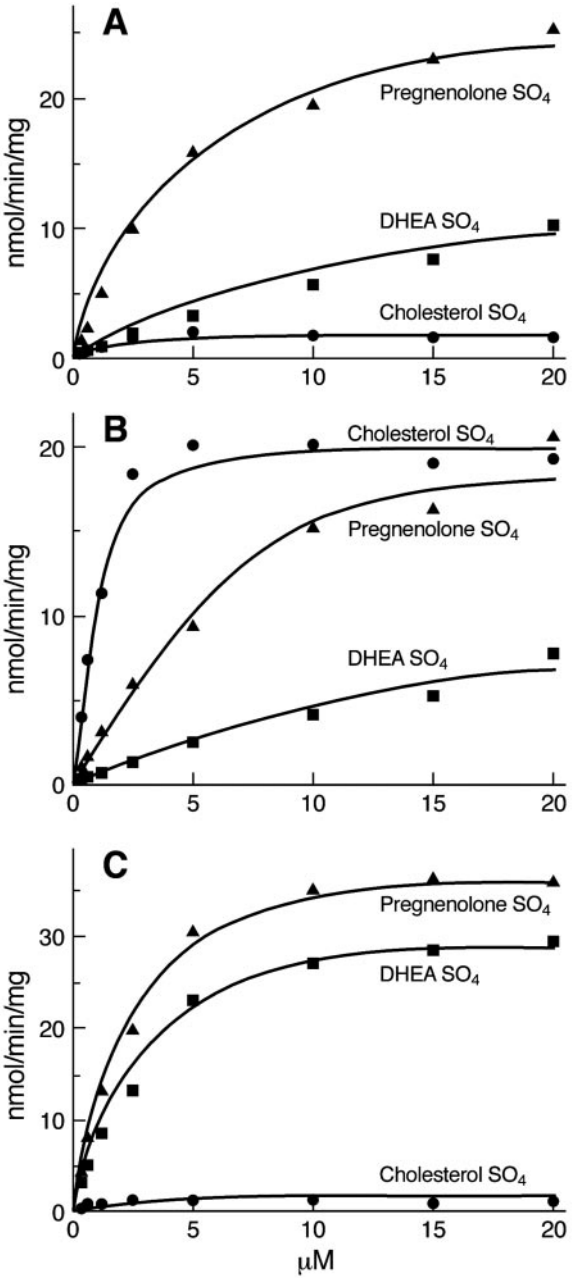

FIG. 4. Saturation analyses of bacterially overexpressed and affinity-purified human hydroxysteroid sulfotransferase SULT2B1a (A), SULT2B1b (B), and SULT2A1 (C). Substrates (μM), PAPS cofactor (0.1 mM), and purified proteins (0.1–4.0 μg) were incubated in Tris buffer (pH 7.5) containing $MgCl_2$ (5 mM), hydroxypropyl-β-cyclodextrin (0.2 mM), and ethanol (4%) at 37 C for 5 min, and the sulfonated products were isolated by thin-layer chromatography. Each *point* represents the average of duplicate determinations.

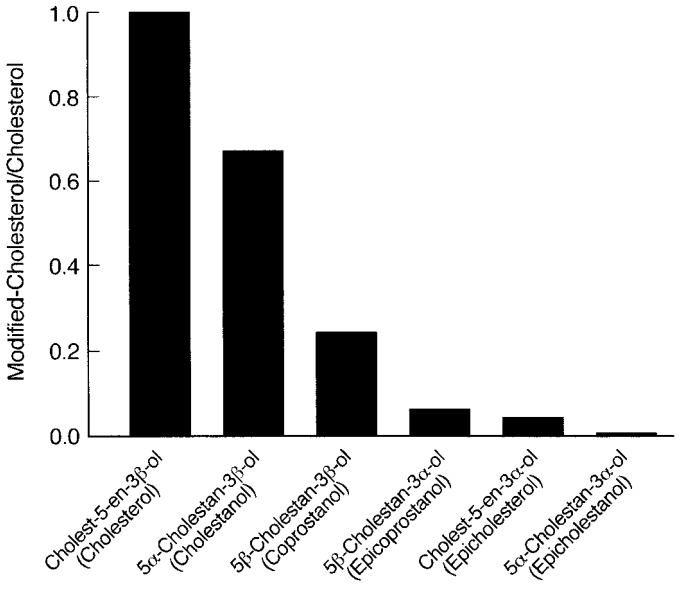

FIG. 5. Sulfotransferase activity of bacterially overexpressed and affinity-purified human hydroxysteroid sulfotransferase, SULT2B1b, using cholesterol or modified-cholesterol as substrate (20 μM). Results are expressed as the amount of modified-cholesterol sulfate formed (nmol/min·mg protein) relative to the amount of cholesterol sulfate formed (nmol/min·mg protein). [Reproduced by permission of N. B. Javitt, unpublished data.]

solic sulfotransferases (109, 115) are completely conserved (*cf.* Fig. 6). Furthermore, regions interacting with the 5′ (5′PB) and 3′ (3′PB) phosphate groups of PAPS are also highly conserved (*cf.* Fig. 6).

The human SULT2B1 subtypes, which differ only at their amino termini, are produced by utilization of an alternative exon 1 (118). The functional significance of the extended carboxy-terminal end of the SULT2B1 subtype is not presently appreciated. One speculation is that the proline-enriched carboxy-terminal region might play a role in protein-protein interactions (127). Interestingly, the last 52 amino acids from the carboxy-terminal end, which is common to both isoforms, can be removed without producing a significant change in catalytic activity of either isoform (131a). On the other hand, removal of the first 23 amino acids from the amino-terminal end, which is unique to SULTT2B1b, results in loss of cholesterol sulfotransferase activity, whereas removal of the 8 amino acids that are unique to SULT2B1a does not alter pregnenolone sulfotransferase activity (131a). It is noteworthy that exon 1B of the human *SULT2B1* gene encodes for only the unique amino-terminal 23 amino acids of the SULT2B1b subtype, whereas exon 1A encodes for the unique amino-terminal 8 amino acids of the SULT2B1a subtype plus 48 additional amino acids that are common to both subtypes (118). Thus, if the gene for human *SULT2B1* employs exon 1B, cholesterol sulfotransferase is synthesized, whereas if the gene employs exon 1A, pregnenolone sulfotransferase is synthesized (131a).

As determined by RT-PCR, the human SULT2A1 and SULT2B1 genes are differentially expressed (127, 132). Human SULT2A1 is robustly expressed in steroidogenic organs (adrenal and ovary), androgen-dependent tissue (prostate), tissues of the alimentary tract (stomach, small intestine, and colon), and the liver; however, it is, notably, not expressed in skin (127). The RT-PCR expression patterns of SULT2B1a and SULT2B1b indicate that SULT2B1b is more widely expressed than SULT2B1a. Importantly, the SULT2B1 subtypes, in contrast to SULT2A1, are vigorously expressed in skin (127). The importance of the observation on the selective expression of SULT2B1 in skin lies in the fact that sulfonation of cholesterol is an essential metabolic step during normal skin development and creation of the barrier (133–135). Differentiation of normal human epidermal keratinocytes is accompanied by an accumulation of cholesterol sulfate, which is accounted for by an increase in cholesterol sulfotransferase activity (136).

Cloning of a mouse SULT2B1b, which is 71% identical with human SULT2B1b, has also been reported (119). Furthermore, similar to human SULT2B1b, the mouse ortholog has a preference for cholesterol (author's unpublished data). Recently, the isolation of a sulfotransferase from rat skin was reported to be active against substrates with a Δ_5 double bond such as cholesterol, whereas androgens, estrogens, corticosteroids, simple phenols, and 3,4-dihydroxyphenylalanine did not serve as substrates (137). The rat cholesterol SULT has an apparent molecular weight of 40,000, which compares with a calculated molecular weight of 38,404 for mouse SULT2B1b and 41,304 for human SULT2B1b. For comparison, human, mouse, and rat SULT2A1 proteins have molecular weights of 33,777; 33,342; and 32,961, respectively. Thus, based on molecular weight and substrate specificity, the recently isolated rat sulfotransferase would appear to belong to the SULT2B1 subfamily (131a).

C. Transcriptional regulation of steroid sulfotransferases

Although a large number of steroid sulfotransferase genes have now been cloned in several species, there has been little information forthcoming regarding their transcriptional regulation, with the exception of rat DHEA sulfotransferase (SULT2A1), a subject on which two papers have emerged (138, 139). Rat SULT2A1 is selectively manifest in liver, in which expression is strongly repressed by androgens (140). In this regard, hepatocyte nuclear factor-1 (HNF1) and CCAAT/enhancer-binding protein (C/EBP) response elements play pivotal roles (138). Regarding androgen repression, a negative androgen response region in the rat SULT2A1 promoter has been mapped. Androgenic repression of the rat SULT2A1 gene requires the presence of OCT-1 and C/EBP elements that map to specific promoter locations. Furthermore, the negative androgenic regulatory effect may be exerted indirectly through transcriptional interference of OCT-1 and C/EBP rather than via a direct interaction of the androgen receptor with DNA (138).

Human SULT2A1 sulfonates bile acids (122). Thus, it is of interest that the bile acid chenodeoxycholic acid is a strong inducer of the rat SULT2A1 gene (139). Furthermore, the inducing effect is mediated through the bile acid-activated farnesoid X receptor (FXR), a member of the nuclear receptor superfamily. Ligand-activated FXR forms a heterodimer with the 9-*cis* retinoic acid receptor (RXR) and binds to a defined FXR/RXR promoter position to regulate rat

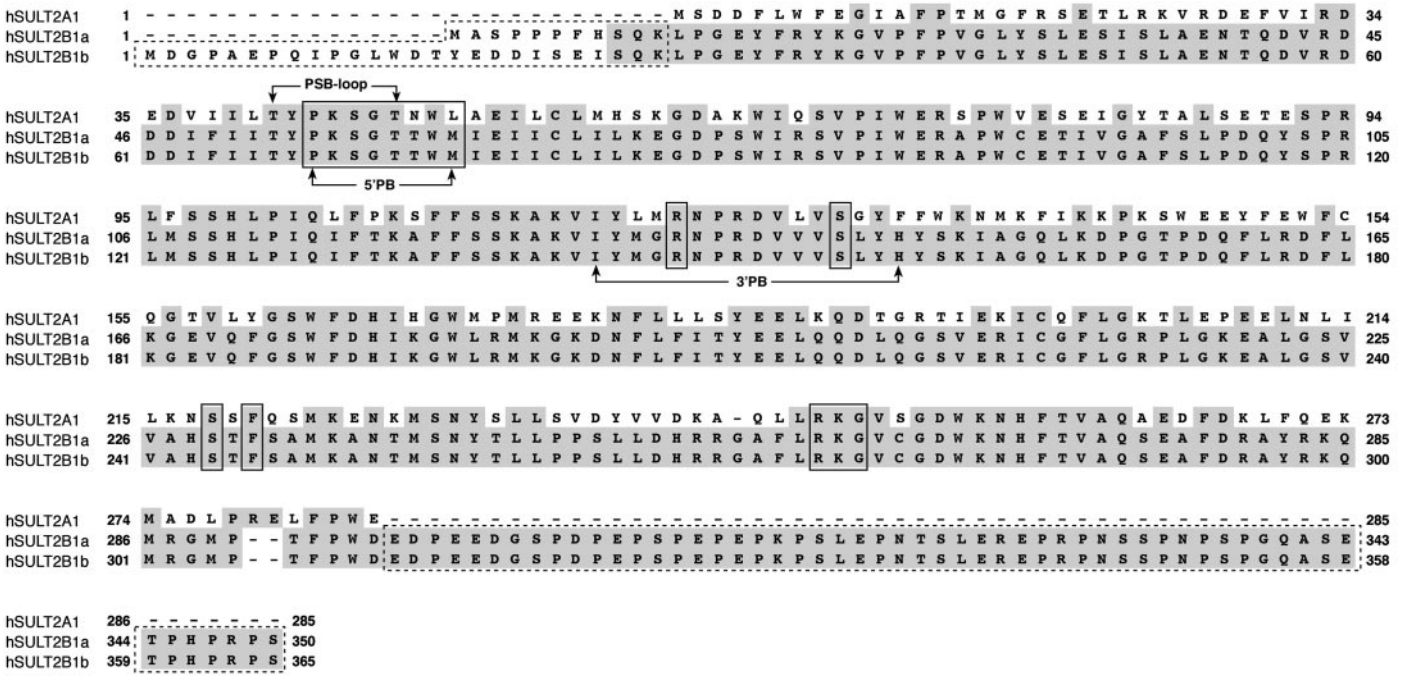

FIG. 6. Amino acid sequence alignment of human (h) SULT2A1, SULT2B1a, and SULT2B1b. The unique amino- and carboxy-terminal ends of hSULT2B1a and hSULT2B1b are *outlined by dashed boxes*. *Solid boxes* locate conserved residues important in cofactor binding. *Arrowheads* locate the highly conserved nucleotide-binding motif (PSB) as well as the 5′ phosphate (5′PB)- and 3′ phosphate (3′PB)-interacting regions. Identities are *shaded*.

SULT2A1 gene expression (139). The same tissues in the rat (liver, small intestine, colon, and adrenal cortex) express SULT2A1 and FXR, and as a result of bile acid sulfonation, SULT2A1 may play a significant role in cholesterol removal (139).

D. Structural analysis of steroid sulfotransferases

1. Crystallography. The first steroid sulfotransferase structure to be solved was that of mouse estrogen sulfotransferase (SULT1E1; Ref. 109). Mouse SULT1E1 was co-crystallized with PAP (desulfonated PAPS) followed by soaking with estradiol. This enabled the structural features important for both cofactor and substrate binding to be determined. Subsequently, the crystal structures of two other cytosolic sulfotransferases were determined, *i.e.*, human dopamine/catecholamine sulfotransferase (SULT1A3; Refs. 110 and 111) and human hydroxysteroid sulfotransferase (SULT2A1; Ref. 112). Importantly, key structural elements are completely conserved and superimposable in all three enzymes. Furthermore, the structural features of the cofactor binding site, *i.e.*, the PSB loop, as well as the 5′-phosphate and 3′-phosphate binding sites (*cf.* Fig. 6), are conserved in the three crystal structures. To date, however, the crystal structure of only the mouse estrogen sulfotransferase has been solved in the presence of substrate (109). Recently, the crystal structure of the human SULT1E1-PAPS complex was solved, which represents the first structure containing the active sulfonate donor for any sulfotransferase (113). The latter study has revealed a crucial reaction mechanism involving a 3′-phosphate-serine[137] interaction. This helps to regulate the action of lysine[47] in controlling the dissociation of the 5′-sulfate group form PAPS (113).

2. Mutagenesis. In addition to crystallization, important structural information has also been garnered from studies utilizing site-directed mutagenesis to investigate a specific functionality. For instance, the structural basis for the high substrate specificity of SULT1E, which sulfonates the 3-hydroxyl group of phenolic steroids such as estrone and estradiol but not the 3-hydroxyl group of neutral steroids such as pregnenolone or DHEA (141), has been examined. It was determined that the 3β-hydroxyl group of DHEA is excluded from the active site by steric hindrance of a tyrosine at position 81with the C-19 methyl group of DHEA creating a substrate gating phenomenon (142). In another case, the chirality exhibited by the two guinea pig SULT2A1 isoforms that demonstrate strict stereospecificity for either a 3α-hydroxyl or a 3β-hydroxyl group (143) was investigated. It was determined that the type of residue at position 51 has a significant ability to regulate this stereoselectivity, *i.e.*, if the residue is an asparagine, α-activity predominates, whereas if an isoleusine is in that position, β-activity prevails (144). It is anticipated that solving the crystal structure of additional steroid sulfotransferases in the presence of their substrates will further elucidate underlying structural principles that determine substrate specificity.

Steroid sulfotransferases are generally homodimers in solution, and the structural elements responsible for the dimerization have been identified (145). A 10-residue segment near the carboxy terminus of SULT-proteins forms a hydrophobic zipper-like structure further enforced by ion pairs at both ends. This amino acid stretch, which includes a critical valine,

is conserved as a KxxxTVxxxE motif in nearly all cytosolic sulfotransferases and appears to be the common protein-protein interaction motif mediating dimerization phenomena (145).

E. Biology

There continues to be a broad interest in exploring the potential biological role of steroid sulfonation in brain development and function (146, 147). This is particularly so for the sulfonates of DHEA (148–151) and pregnenolone (152–155). These latter studies are in addition to the well-established nongenomic actions of the sulfates of DHEA and pregnenolone on the $GABA_A$ and *N*-methyl-D-asparate receptors (156). Importantly, steroid sulfotransferase activity and proteins have now been detected in brain tissue (157–160).

In humans, DHEA, together with its sulfate, comprises the most abundant steroid in the circulation; however, the physiological significance of this phenomenon remains elusive. The plasma concentrations of DHEA and DHEA sulfate, which are produced by the zona reticularis of the adrenal cortex (161), undergo a dramatic age-related decline throughout adulthood from their postpubertal peak (162, 163). On the other hand, antiparallel to the decline of DHEA and DHEA sulfate, plasma cortisol levels show an increase (164). Chiefly as a result of this phenomenon, DHEA and DHEA sulfate have been implicated in a variety of physiological systems as well as pathophysiological disorders (165). There is a particular interest in these steroids in the aging process (165, 166). As a result, their use as potential therapeutic or counter-aging agents continues unabated, unfortunately, however, with conflicting results (165).

There also continues to be an interest in potential roles for steroid sulfotransferases in the induction and maintenance of endocrine-dependent cancers. This pertains principally to breast cancer (167–171) and carcinoma of the prostate gland (172). In theory, the formation and hydrolysis of steroid sulfates such as estrone sulfate in breast tumors or testosterone sulfate in prostate tumors could be an important mechanism regulating the availability of unconjugated steroids to interact with cognate nuclear receptors. Because only the unconjugated steroid hormone has growth-promoting activity, abnormal regulation of sulfotransferases has pathological implications. Furthermore, specific steroid hormone sulfotransferase transfection experiments have demonstrated effective reductions in cellular responses to physiological hormone concentrations (168, 172). Work in this area has led to the proposal that a potential target for antitumor therapy could be steroid sulfatase, the enzyme responsible for the hydrolysis of steroid sulfates (171). Although these various reports are of interest, there is no convincing evidence to support an etiological or pathophysiological role for any steroid sulfotransferase or sulfatase in endocrine-dependent tumorigenesis.

The biological effect produced by certain drugs and xenobiotics by targeting specific steroid sulfotransferases has been an interesting recent development (173, 174). By blocking the formation of a steroid hormone sulfate, thus increasing availability of the unconjugated form to interact with its cognate nuclear receptor, a xenobiotic can, in effect, exert an indirect hormonal action. Furthermore, this effect may not necessarily be associated with a significant change in the circulating steroid hormone level but may simply reflect a local expression in hormone-sensitive tissue.

A powerful technique for evaluating the biological significance of specific genes is to selectively inactivate the gene in question in an animal model. For example, targeted gene disruption has been used to evaluate estrogen sulfotransferase (175). It has been determined that disruption of the mouse *SULT1E* gene led to structural lesions in the adult male testis. Although knockout males are initially fertile and phenotypically normal, they eventually develop age-dependent Leydig cell hypertrophy/hyperplasia and seminiferous tubule damage. Additionally, older mice have reduced sperm motility and produce smaller litters compared with age-matched wild-type males (176). Disruption of the mouse *SULT1E* gene in female mice results in a significant reduction in fertility, although the nature of the defect is not known (176). It is hoped that this approach, which is important for evaluating the developmental and physiological significance of sulfoconjugation, will expand to involve other steroid sulfotransferase genes.

V. Sulfonation and Thyroid Function

A. General

The raison d'être of the thyroid gland is to produce the hormones T_4 and T_3, a process that involves the convergence of intrathyroidal iodine metabolism and protein synthesis. Thyroglobulin is iodinated at specific tyrosine residues followed by precise coupling steps, which subsequently lead to the formation of peptide-linked tri- and tetraiodothyronines. Eventually, proteolysis occurs with the release of T_4 and T_3 for secretion into the circulation while any residual iodine is recycled (177). Sulfonation plays an intimate role in thyroid physiology: it is important for both synthesis (178) and metabolism of thyroid hormones (179). In addition, TSH is subject to posttranslational modification by sulfonation (180, 181).

B. Sulfonation of thyroglobulin

Thyroglobulin is the major protein produced by the thyroid gland and the macromolecular precursor of thyroid hormones (182). During the course of synthesis and processing, thyroglobulin undergoes extensive posttranslational modification, including iodination (183), glycosylation (184), phosphorylation (185), and sulfonation (186). Modification of thyroglobulin by sulfonation involves both carbohydrate units and peptide chains. For instance, galactose-3-sulfate and *N*-acetylglucosamine-6-sulfate have been identified in the asparagine-linked complex of human thyroglobulin (187). Galactose-3-sulfate occurs primarily as a terminally linked residue, where it forms a novel-capping group. Additionally, tyrosine residues in the protein core of thyroglobulin are also subject to sulfonation (188). The fact that tyrosine sulfonation is a common posttranslational modification of thyroglobulin found throughout the vertebrate phylum suggests that it was acquired at an early stage in

thyroid evolution (188). Interestingly, it has been demonstrated that TSH down-regulates thyroglobulin sulfonation, particularly on tyrosine residues (189).

The biological role of the sulfonated sugars in thyroglobulin (both the terminally capped galactose-3-sulfate as well as the more internally located *N*-acetylglucosamine-6-sulfate) remains uncertain. It has been speculated that sulfate groups linked to oligosaccharide chains or to tyrosine residues of thyroglobulin might be involved in protein-protein recognition and have implications for the proper folding of thyroglobulin (189). Furthermore, sulfated tyrosines in thyroglobulin may be involved in hormonogenesis, and the control of tyrosine sulfonation by TSH could be related to this process. The sulfate group could act to modify the electronic environment of the aromatic ring of tyrosine whereby it becomes more reactive for iodination and coupling; the sulfate group could be a recognition signal for thyroid peroxidase or the iodotyrosine donor (189). In a recent study (178), thyroid hormone synthesis was found to correlate with the sulfotyrosine content of thyroglobulin. Interestingly, a consensus sequence (Asp/Glu-Tyr) is similarly involved in thyroid hormone synthesis (190) and the sulfonation of tyrosine (191). Based on this observation, it has been proposed that thyroglobulin sulfotyrosines may act either as a signal for iodination or the coupling reaction (the latter is thought to be the more likely possibility) by inducing an interaction between thyroid peroxidase and thyroglobulin (178). Understanding the exact details of this complex system is a work in progress.

C. Sulfonation of thyroid hormones

T_4, the main secretory product of thyroid follicular cells, is converted in extrathyroidal tissues to T_3, the biologically active form of thyroid hormone (192). T_4 is activated by outer-ring deiodination (ORD) to T_3, and both T_4 and T_3 are inactivated by inner-ring deiodination (IRD) to rT_3 and diiodothyronine (DIT), respectively (Fig. 7). ORD and IRD are carried out by the enzyme type I iodothyronine deiodinase, an enzyme found primarily in the liver, kidney, and thyroid (193). Importantly, T_4 and T_3, as well as other iodothyronines, are subject to sulfoconjugation (194). Sulfonation of the phenolic hydroxyl group of T_4 and T_3 (Fig. 7) is carried out by a number of tissues including human liver (193). The sulfates of T_4 and T_3 are normal components of human serum, in which levels are subject to physiological and pathophysiological conditions (195, 196). T_3 sulfate is detectable in the fetal circulation, and its concentration increases with fetal age (197). Although T_3 sulfate does not bind to nuclear receptors and therefore lacks intrinsic biological activity (198), sulfonation of iodothyronines is nevertheless an important metabolic step in determining their disposal (179). For instance, the majority of normal T_3 disposal occurs via T_3 sulfate formation (199). It has long been known that sulfonation facilitates the deiodination of iodothyronines (200). Type I deiodination of T_3 sulfate in human liver homogenates occurs at a rate that is 30-fold higher as compared with unconjugated T_3, and HepG2 cells, which are deficient in T_3-sulfonating activity, are unable to deiodinate T_3 (201). It has been suggested that the function of sulfonation is to inactivate thyroid hormone so that iodine can be reused for thyroid hormone synthesis (202). This intriguing idea is supported by the demonstration that the sulfonation of T_4 and T_3, as well as other metabolites, strongly promotes hepatic deiodination (202). It is concluded that sulfonation simultaneously accelerates the IRD of both T_4 and T_3 (inactivation) and blocks the ORD of T_4 (activation) and thus represents a crucial step in

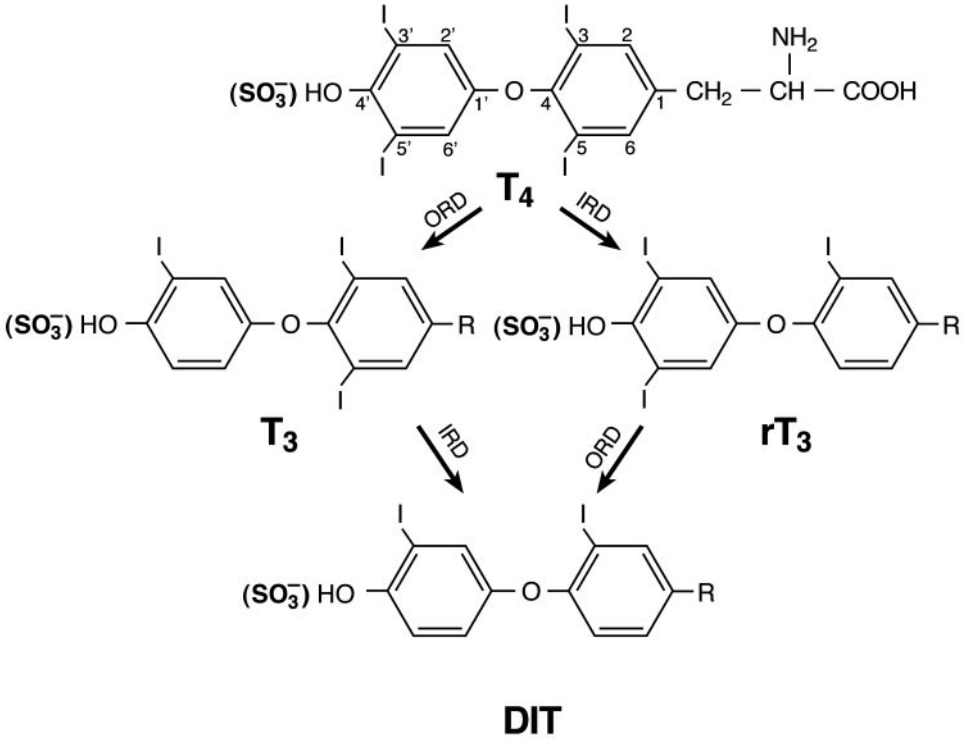

FIG. 7. Stepwise deiodination of T_4 by ORD to T_3 and by IRD to rT_3 and further by IRD of T_3 and ORD of rT_3 to 3,3′-DIT. Potential sulfonation acceptor sites are shown by (SO_3^-).

the irreversible inactivation of thyroid hormones (203). On the other hand, if for some reason (*e.g.*, propylthiouracil treatment) iodothyronine deiodinase activity is low, then T_3 sulfate becomes a reversible pathway in that biologically active unconjugated T_3 can be regenerated by the action of a tissue sulfatase (193). Akin to the sulfonation of thyroglobulin, the metabolism of iodothyronines by sulfoconjugation is an ever-evolving subject.

D. Sulfonation of TSH

The sulfonation of TSH will be covered in *Section VII*, which deals with the sulfonation of peptide hormones.

E. Sulfotransferases

The sulfotransferase enzymes that modify thyroglobulin, involving both the carbohydrate chains and the core peptide, are membrane-associated enzymes found in the Golgi complex. The polypeptide chain of thyroglobulin is synthesized in the endoplasmic reticulum, in which carbohydrate chain synthesis is also initiated. On formation of stable dimers, the nascent protein enters the Golgi system, where the carbohydrate units are completed and sulfonation takes place (177).

1. Enzymes sulfonating the carbohydrate chains of thyroglobulin. Galactose 3-*O*-sulfotransferase activity involved in the formation of the galactose-3-sulfate capping groups present in the asparagine-linked oligosaccharides of thyroglobulin is located in the Golgi compartment (204). Four human galactose 3-*O*-sulfotransferases sharing approximately 40% identity have been cloned that carry out the sulfonation of different acceptor substrates (205). Of the four galactose 3-*O*-sulfotransferases, one was found to be highly expressed in the thyroid gland and is considered responsible for the formation of galactose-3-sulfate in $\beta 1 \rightarrow 4$ linkage to *N*-acetylglucosamine attached to both *N*-glycans, and core 2-branched *O*-glycans synthesized in the thyroid (206). Although several 6-*O*-sulfotransferases, including *N*-acetylglucosamine 6-*O*-sulfotransferases, have been cloned (87), the sulfotransferase utilizing thyroglobulin (187) as an acceptor has not been identified.

2. Enzymes sulfonating tyrosine residues of thyroglobulin. Protein tyrosine sulfonation is a widespread posttranslational modification of many secretory and membrane proteins (7, 8). Tyrosylprotein sulfotransferase is a 50,000- to 54,000-molecular-weight integral membrane protein of the *trans*-Golgi network that is found in essentially all tissues (207). Two distinct human tyrosylprotein sulfotransferase genes have been identified and the cDNAs cloned (106, 107, 208). The two tyrosylprotein sulfotransferases are 64% identical, with most of the variation between the two proteins found within the ends of the amino and carboxy termini (107). Although Northern analysis revealed that both genes were expressed in all the human tissues examined, expression by the thyroid gland has not been specifically investigated. Whether the two enzymes are functionally redundant or whether they might utilize preferred substrates is not known.

3. Enzymes sulfonating thyroid hormones. In contrast to the Golgi-associated sulfotransferases that act on macromolecules, the enzymes that utilize iodothyronines as substrates are part of a large family of SULTs. Human SULTs with documented activity toward iodothyronines include SULT1A1 (thermostable phenol SULT; Refs. 209–213), SULT1A3 (thermolabile phenol SULT; Refs. 209 and 212), SULT1B1 (214, 215), SULT1E1 (estrogen SULT; Refs. 216 and 217), SULT1C1 (218), and SULT2A1 (hydroxysteroid SULT; Ref. 216). These multiple human SULT isoforms have overlapping specificities involving simple phenols, dopamine, estrogens, hydroxysteroids, as well as iodothyronines. A recent *in vitro* study (219) examined which SULT form makes a major contribution to the metabolism of T_3 by comparing five different overexpressed and purified human SULTs, *i.e.*, SULT1A1, SULT1A3, SULT1B1, SULT1E1, and SULT2A1. Of the five enzymes, SULT1B1 demonstrated the lowest Michaelis-Menten constant (K_m) and the highest general rate constant (k_{cat})/K_m for T_3; whereas SULT1A1 preferred the simple phenol *p*-nitrophenol, SULT1A3 preferred dopamine, SULT1E1 preferred estradiol-17β, and SULT2A1 preferred DHEA (219). These results are consistent with the previous characterization of these SULT forms. Although the actual *in vivo* role of an individual SULT form in the metabolism of iodothyronines remains to be determined, this *in vitro* study clearly suggests that SULT1B1 is the principal SULT form involved in the metabolism of T_3. Furthermore, the content of SULT1B1 in human liver highly correlates with T_3-sulfonating activity (219), adding further support for SULT1B1 being the principal hepatic iodothyronine-sulfonating enzyme. SULT1B1 is expressed in the small intestine and colon, as well as in the liver; however, expression by thyroid tissue was not determined (215). SULT1C1, which was not included in the previous SULT comparison study, also sulfonates iodothyronines and is expressed in the thyroid gland, as well as in a number of other tissues (220). During fetal brain development, SULT activity with DIT as substrate correlated with SULT1A1 expression, suggesting that this SULT form is primarily responsible for the sulfonation of DIT (221). In summary, the involvement of a specific SULT form expressed in a specific tissue in the metabolism of iodothyronines remains to be fully elucidated.

VI. Sulfonation of Catecholamines

A. General

Conjugation represents a major metabolizing mechanism for catecholamines to the extent that approximately 84% of total epinephrine, 73% total of norepinephrine, and 97% of total dopamine circulate in a conjugated form (222–224). In man, the conjugated form of catecholamines is almost entirely sulfoconjugation, which is in contrast to rats, in which glucuronidation predominates (223). In addition, norepinephrine is methylated extraneuronally by the enzyme catechol *O*-methyltransferase to produce normetanephrine, and 77% of total circulating normetanephrine is also found in a sulfoconjugated form; again, this is in contrast to the rat, in which 63% of circulating normetanephrine is glucuronidated (225).

Free catecholamines exhibit a short plasma half-life of 1–3 min, in contrast to catecholamine sulfates, which have a

plasma half-life of 3–4 h (226). Normotensive recumbent subjects exhibit an early (~2300 h) nocturnal increase in the plasma concentration of the sulfates of dopamine, norepinephrine, and epinephrine (227); however, an explanation for this nocturnal rise has not been forthcoming. Catecholamines are sulfoconjugated predominantly at carbon-3 of the phenyl ring (Fig. 8), and the phenol sulfotransferase (SULT1A3) that carries out this reaction has the greatest affinity for dopamine, followed by norepinephrine and epinephrine (228). For plasma dopamine, which is more than 95% sulfonated, both the 3-*O*- and 4-*O*-sulfate isomers are present, with the 3-*O*-sulfate being in greater abundance by an order of magnitude (229).

B. Sources of catecholamine sulfates

Sulfonation of catecholamines occurs in platelets and other peripheral tissues (230–232). It may also occur within neural tissue, but this possible source remains controversial (233). Three potential sources of plasma catecholamine sulfates are platelets, the intestinal barrier, and the liver (234). Additionally, any tissue in which the whole enzymatic process leading to sulfoconjugation of catecholamines can take place is a conceivable source (235). In an early study (236), regional venous measurement of free and sulfoconjugated catecholamines in patients with essential hypertension indicated that catecholamines were released in free form from the adrenal medulla and subsequently sulfoconjugated during venous passage, presumably by platelet phenol sulfotransferase. However, in a subsequent study (237) of patients with severe platelet deficiency, it was determined that platelet phenol sulfotransferase is not indispensable for the sulfoconjugation of plasma catecholamines and that this process must also be occurring elsewhere. Interestingly, the activity of the sympathetic nervous system is not considered to be a major source of sulfoconjugated catecholamines (235). In a study (238) involving patients with pure autonomic failure, it was concluded that dopamine sulfate is derived mainly from nonneural sources, such as the gastrointestinal tract, rather than from the sympathoadrenomedullary system. Lack of importance of the liver in the sulfoconjugation of dopamine is suggested by the finding that patients who are anhepatic and awaiting liver transplants have normal levels of plasma dopamine sulfate (232). Furthermore, the phenol sulfotransferase isozyme expressed in hepatic tissue is the thermostable (SULT1A1) form and not the thermolabile (SULT1A3) form (239), with the latter form representing the phenol sulfotransferase that is primarily responsible for catecholamine sulfonation (240). In a recent detailed study (241), it was concluded that both dietary intake and synthesis and metabolism of endogenous dopamine, especially in the gastrointestinal tract, are important in determining plasma dopamine sulfate levels. It was determined that most of the body's total dopamine sulfate production results from the conjugation of dopamine in the gastrointestinal tract (derived from dietary and/or endogenous sources of tyrosine, levo-dihydroxyphenylalanine, and dopamine). This is consistent with the finding that the principal phenol sulfotransferase isozyme that acts on catecholamines (SULT1A3) is located in tissues of the gastrointestinal tract (240). Thus, during a fast, the rate of entry of dopamine sulfate into plasma is equivalent to the rate of dopamine production in the gastrointestinal tract (241).

	R_1	R_2
DA	H	H
NE	OH	H
E	OH	CH_3

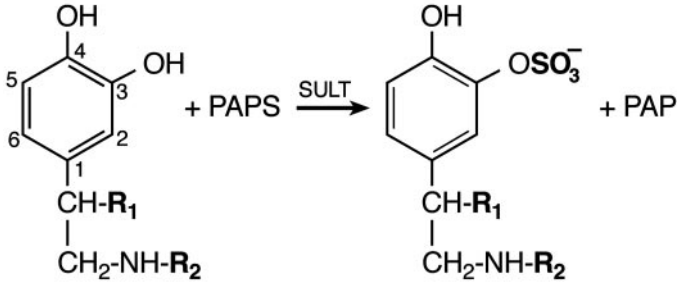

FIG. 8. Sulfonation of catecholamines (DA, dopamine; NE, norepinephrine; E, epinephrine) by phenol sulfotransferase SULT1A3.

C. Catecholamine sulfotransferases

As noted in *Section III*, which deals with SULTs, the subfamily designated as SULT1 is principally involved in the sulfonation of xenobiotics, drugs, and phenolic compounds. At least seven members of the SULT1 subfamily (1A1, 1A2, 1A3, 1B1, 1C1, 1C2, and 1E1) are known to be expressed in humans (91). Although all members of the SULT1 subfamily demonstrate overlapping specificity, it is SULT1A3 that exhibits selectivity toward catecholamines (242). SULT1A3 was originally referred to as the thermolabile form or M-form of phenol sulfotransferase (243, 244). SULT1A1, on the other hand, which was originally referred to as the thermostable form or P-form of phenol sulfotransferase, acts on simple phenols, phenolic drugs, and xenobiotics (242–244). SULT1A2 is closely related to SULT1A1 at the amino acid sequence level, but its physiological role and expression pattern are not well understood (245, 246). As recently reported (94), SULT1A1, SULT1A2, and SULT1A3 also sulfonate catechol estrogens, although far less efficiently than SULT1E1. These isoforms represent paralogs whose genes are clustered on chromosome 16p11.2–12 (247–250).

SULT1A3 has been cloned and characterized from several human tissues (251–253). Although the SULT1A subfamily members have distinct substrate specificities, their high level of amino acid sequence homology has made specific tissue localization by immunocytochemistry somewhat difficult. To circumvent this problem, a 198-bp fragment from the 3′-end of the untranslated region (UTR) of the SULT1A3 cDNA has been used to develop specific antisense and sense riboprobes for use in hybridization histochemistry. Based on this technique, it was determined that SULT1A3 is expressed within the gastrointestinal tract and by bronchial epithelial cells. Expression in other tissues such as liver and breast, however, was found to be low (254). The crystal structure of

SULT1A3 with a sulfate bound at the active site has been determined at 2.4-Å resolution (110).

D. Physiology and clinical significance of catecholamine sulfonation

The formation of catecholamine sulfates is an important pathway that leads to their inactivation (255). That is, sulfonated catecholamines are neither agonistic nor antagonistic ligands for α- and β-adrenergic or dopaminergic receptors (224, 256–259). One possible exception to catecholamine sulfate inactivity has been the finding that angiotensin-induced stimulation of aldosterone secretion by cultured bovine adrenal cells is similarly inhibited by dopamine and dopamine-3-*O*-sulfate (260). However, because subsequent studies regarding this observation have not appeared, it remains of questionable significance.

Whereas oxidation and transmethylation are irreversible metabolic reactions, sulfonation is a potentially reversible step, *i.e.*, biologically inactive catecholamine sulfates can be reactivated by sulfohydrolysis engendering catecholamine sulfates as a potential source of active hormones (233). Plasma levels of sulfoconjugated catecholamines were noted to be higher in patients with hypertension (261) and atherosclerosis (262), although the significance of these findings is speculative. In our present state of knowledge, the role of catecholamine sulfonation in the pathophysiology of essential hypertension and chronic heart failure is controversial and poorly understood (233).

Although no genetic disorder producing a totally defective SULT has been described in humans, substantial variation in the expression of several SULTs is known to occur (240). For instance, genetic polymorphism of *SULT1A1* is associated with phenotypic variation in both activity and thermal stability (263). Allelic variants accompanying amino acid changes in SULT1A1 and SULT1A2 have been identified by various laboratories (245, 246, 264–268). In a catalog of 320 single-nucleotide polymorphisms, there were 27 involving SULT1A1 (5 in coding exons) and 20 involving SULT1A2 (3 in coding exons) but only 1 involving SULT1A3 (just upstream of the stop codon in exon 8) (269). The other single-nucleotide polymorphisms involving SULT1A1 and SULT1A2 were located in the 5′- and 3′-flanking regions, the 5′- and 3′-UTRs, and various introns (269). The importance of polymorphisms relates to their potential effect on level of expression as well as the structure and activity of individual enzymes. For instance, examination of specific SULT1A1 and SULT1A2 variants has revealed the existence of significant differences in kinetic properties and thermal stability (246). Furthermore, sequencing of SULT1A1 and SULT1A3 cDNAs from various tissues has revealed substantial heterogeneity in the 5′-UTR, suggestive of alternative splicing and/or tissue-specific promoter activity (270).

VII. Sulfonation of Peptide Hormones

A. General

Tyrosine sulfonation is a widespread posttranslational modification of membrane and secretory proteins found in all metazoan species (8). Tyrosine sulfonation takes place in the *trans*-Golgi and is one of the last processing steps before proteins exit the Golgi complex (7). Although the physiological significance of tyrosine sulfonation is not always well understood, its involvement in protein-protein interactions in a variety of systems appears clearly established. For example, tyrosine sulfonation is required for the optimal interaction between factor VIII and von Willebrand factor (271, 272), between P-selectin glycoprotein ligand-1 and P-selectin (273), between glycoprotein Ibα with von Willebrand factor and α-thrombin (274–276), and between complement factor 4 and the complement subcomponent C1s (277–279).

The α-subunit of human chorionic gonadotropin (HCG), a glycoprotein hormone consisting of heterodimeric α- and β-subunits structurally similar to LH, FSH, and TSH, is also subject to tyrosine sulfonation. Interestingly, this modification of HCG, which occurs close to the carboxy terminus, can modulate biological activity (280). Tyrosine sulfonation is also an important posttranslational modification of certain peptide hormones, such as CCK (281) and gastrin (282), in which it plays an essential role in biological activity.

In addition to the tyrosine sulfonation of peptide hormones, sulfonation can also involve the carbohydrate attachments of glycoprotein hormones. The addition of a sulfonate group onto a saccharide moiety of these glycoproteins creates a unique structural motif with important functional consequences (9). Sulfonation of asparagine-linked oligosaccharides, first described for bovine LH (180), is now known to be a prominent posttranslational modification of human LH and TSH. It is not, however, a significant feature of human FSH and CG (283, 284). Sulfonation of sugar residues on glycoprotein hormones is physiologically important because, in mammals, the circulatory half-life of these hormones is controlled by evolutionarily conserved asparagine-linked oligosaccharides that contain a unique sulfated cap (285). Another pituitary glycoprotein that contains sulfated asparagine-linked oligosaccharides is proopiomelanocortin (POMC), although this is of indeterminate functional significance (286).

B. Neuroendocrine peptides

1. CCK. The neuroendocrine peptide CCK is found throughout the gastrointestinal tract and the central nervous system, where it acts as both a hormone and a neurotransmitter (287). Importantly, sulfonation of the single tyrosine residue located at the seventh position from the carboxy terminus is essential for full biological activity (105). Multiple molecular forms of CCK have been identified in the intestine and in the circulation, ranging in length from CCK-8 to CCK-83 with CCK-33 being the predominant form (288). On the other hand, the predominant form of CCK in the brain and peripheral nerves is CCK-8 (289). CCK stimulates gallbladder contraction and the release of digestive enzymes (290). The multiple molecular forms of CCK appear to have a similar potency on pancreatic acinar cells (291). CCK stimulates pancreatic endocrine secretion and growth (292); it also induces satiety, inhibits gastric emptying and gastric acid secretion, and stimulates intestinal peristalsis (291). The cDNA for human CCK encodes for a 115-amino-acid preproCCK mole-

cule that consists of signal and spacer peptides as well as a short extension at the carboxy terminus (293). The gene for CCK is located on the long arm of chromosome 3, spans 7 kb, and is composed of 3 exons (291, 294). Although the CCK gene is expressed in many tissues, expression is highest in the central nervous system and next highest in the gastrointestinal tract; significant expression also occurs in neuroendocrine tissues such as the adenohypophysis and neurohypophysis (295).

CCK is α-amidated at the carboxy terminus, a characteristic feature in common with gastrin as well as with other peptide hormones (296, 297). Structurally, CCK contains a carboxy-terminal pentapeptide (Gly-Trp-Met-Asp-Phe-NH_2) identical with that of gastrin (Fig. 9), which constitutes the minimum structure necessary for biological activity (291). The similarity between CCK and gastrin suggests that they may have evolved from a common ancestor (292). Furthermore, because of the sequence similarity between CCK and gastrin, each peptide can interact with the receptor for the other peptide. This results in gastrin having slight CCK-like activity and CCK having weak gastrin-like activity (291). CCK exerts its biological effect by binding to a specific target tissue receptor, and, importantly, receptor binding is completely dependent on sulfonation of a precise ligand residue (298). On the other hand, the binding of gastrin to the gastrin receptor is only slightly influenced by sulfonation (292). Furthermore, CCK is a fully sulfonated peptide (290), in contrast to gastrin, which is only about half-sulfonated (282).

For CCK isoforms to specifically bind to CCK receptors, the peptides must consist of at least the carboxy-terminal seven amino acids, and full potency is not achieved unless the tyrosine residue at position 7 from the carboxy terminus is sulfonated (Fig. 9). The unsulfonated form of CCK is approximately 1000-fold less active than the sulfonated form (298). There are two CCK receptors: CCK-A and CCK-B (299, 300). The CCK-A receptor has a 500-fold higher affinity for the sulfonated form of CCK over that of the unsulfonated form (299, 301). On the other hand, sulfonation of CCK is not required for binding to the CCK-B receptor, which also serves as the gastrin receptor (see below). Cloning of the human CCK-A receptor has reveled that it belongs to the superfamily of G protein-coupled receptors characterized by seven transmembrane domains with amino-terminal extracellular and carboxy-terminal intracellular loops (300, 302, 303). The CCK-A receptor cDNA, when expressed in transfected cells, demonstrates high affinity for sulfonated CCK but a much lower affinity for the unsulfonated form of CCK (304). Mutational analysis has identified methionine 195 of the CCK-A receptor as a putative amino acid in the interaction with the aromatic ring of the sulfated tyrosine residue in CCK, an interaction essential for CCK-dependent transition of the CCK-A receptor to a high-affinity state (305). Additionally, arginine 197 of the CCK-A receptor is involved in an ionic interaction with the sulfate group of CCK (306). A mutated arginine 197 CCK-A receptor was, respectively, approximately 1500- and 3200-fold less potent than wild-type CCK-A receptor in activation of G proteins and induction of inositol phosphate production, a finding consistent with a 500-fold lower potency and 800-fold lower affinity of unsulfonated CCK for the wild-type CCK-A receptor relative to sulfonated CCK (306). CCK-A receptors mediate gall bladder contraction, pancreatic growth, and enzyme secretion, as well as delay gastric emptying and relaxation of the sphincter of Oddi (307). CCK-A receptors are also located in the anterior pituitary gland and in areas of the midbrain (295).

2. Gastrin. Like CCK, gastrin is a regulatory peptide with both endocrine and neurotransmitter functions (295). The two principal biological roles for gastrin are regulation of acid secretion from gastric parietal cells and stimulation of mucosal growth in the acid-secreting part of the stomach (308). Gastrin was purified from antral mucosa G cells and was identified as two 17-amino-acid peptides, one of which was sulfonated at a tyrosine six residues from the carboxy terminus (Fig. 9 and Refs. 309 and 310). Like CCK, gastrin is α-amidated at the carboxy terminus (309, 310), and removal of the terminal amide results in almost complete loss of biological activity (311). As noted above, gastrin and CCK exhibit a common carboxy-terminal pentapeptide that forms a minimal structure necessary for receptor binding and biological activity (292, 311). Another feature in common with CCK is that gastrin exists in a number of biologically active

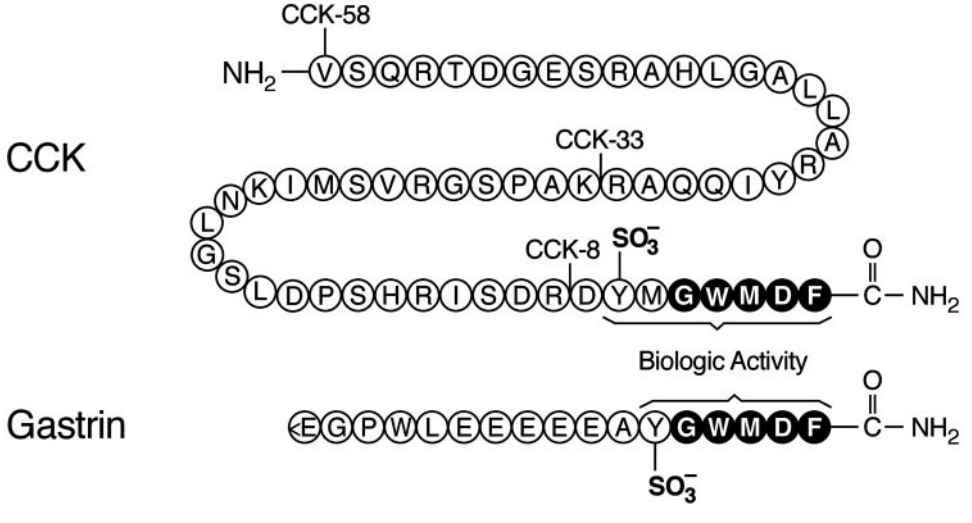

FIG. 9. Structures of CCK and gastrin using single-letter amino acid symbols. Primary sequence of the most prominent mammalian forms of CCK, *i.e.*, CCK-58, CCK-33, and CCK-8 are indicated, as are the tyrosine sulfate located at the seventh position from the α-amidated carboxy terminus. The major sulfonated form of gastrin (17-II) is shown with the tyrosine sulfate located at the sixth position from the α-amidated carboxy terminus. *Filled circles* and *reverse type* delineate an identical carboxy-terminal pentapeptide sequence shared by both peptides. (<E) indicates pyroglutamic acid.

forms containing various amino-terminal extensions that influence potency (292). Gastrin peptides are derived from an 80-amino-acid gastrin precursor molecule, progastrin (312). Of the bioactive amidated forms of gastrin, 80–90% is gastrin-17, with gastrin-34 comprising 5–10%, whereas the rest consists of a mixture (313). Additionally, as a result of a novel processing mechanism involving both gastrin-34 and gastrin-17, a smaller gastrin molecule, *i.e.*, sulfonated gastrin-6, is produced that has a potency similar to gastrin-17 (312). Interestingly, gastrin-6 is completely sulfonated in contrast to gastrin-17, which is only about half-sulfonated (314). The cDNA and gene for human gastrin have been cloned and characterized (315–318). The gastrin gene is located on the long arm of chromosome 17, spans 4.1 kb, and consists of 3 exons. In man, preprogastrin consists of 101 amino acids and is structurally quite similar to preproCCK; furthermore, the active site and structures around major processing sites are conserved (295). In addition to the antral mucosa, which is the overwhelming source of gastrin, the gastrin gene is also expressed in other tissues, including the small intestine, adenohypophysis, neurohypophysis, and gonads (295).

As discussed previously, the receptor for gastrin is the same as the CCK-B receptor. Similar to the CCK-A receptor, the CCK-B/gastrin receptor is a member of the G protein-coupled receptor superfamily that has an equal affinity for both gastrin and CCK (299). The human gastrin receptor cDNA encodes for a 447-amino-acid protein. The gastrin receptor gene, which is located on chromosome 11, is expressed in the stomach, pancreas, gallbladder, and brain (319). Furthermore, the CCK-B receptor is the predominant CCK receptor in brain, and expression is particularly high in the cerebral cortex (320). Sulfonation of gastrin-17 was originally reported to have no physiological effect (309, 311). Subsequent studies, however, reveal that the sulfonation of gastrin significantly increases biological activity (321, 322), a finding consistent with data demonstrating that sulfonation of gastrin increases affinity for its receptor by 19-fold (323). Nevertheless, the effect of sulfonation on receptor affinity is quite modest for gastrin when compared with that for CCK. For instance, the sulfonation of CCK-8 increases its affinity for the CCK-A receptor by 500- to1000-fold, whereas the sulfonation of gastrin-17 increases its affinity for the CCK-B/gastrin receptor by about 20-fold. Furthermore, sulfonated CCK-8 is 70-fold more potent than unsulfonated CCK in interacting with the CCK-B/gastrin receptor (323). Nonetheless, both CCK-A and CCK-B/gastrin receptors are similar in that the affinity of the two receptors for CCK and gastrin is increased by hormone sulfonation, and each receptor has a similar relationship to the affinity of the sulfonated and unsulfonated peptide forms. That is, the gastrin receptor has a higher affinity for sulfonated gastrin than sulfonated CCK, and a higher affinity for unsulfonated gastrin than unsulfonated CCK. Likewise, the CCK receptor has a higher affinity for sulfonated CCK than sulfonated gastrin, and a higher affinity for unsulfonated CCK than unsulfonated gastrin (323).

The effect of gastrin sulfonation on biological activity is more variable than the case with CCK sulfonation. Whereas sulfonated gastrin is more potent than the unsulfonated form on pancreatic acini (321–323), gastrin sulfonation appears to have no effect on gastric acid secretion (309, 324). The reason for this difference in biological effects is unclear; it is not felt to be due to any significant difference in gastrin receptors in the stomach *vs.* the pancreas (323). Some of the physiological difference could be species-related, although the lack of an effect of gastrin sulfonation on gastric acid secretion has been noted in animal as well as human studies. There is also uncertainty regarding the effect of sulfonation on gastrin metabolism. In one study involving humans (325), the half-life of sulfonated gastrin-17 was 2–5 times greater than that of the unsulfonated form, whereas in another study (324), sulfonated and unsulfonated gastrin had similar rates of metabolism.

3. Other peptides. The sulfonation of two other peptides, in which the sulfate moiety influences biological activity, is known, namely leu-enkephalin (326) and angiotensin II (327). In each case, biological activity is significantly reduced by sulfonation. For example, sulfoconjugated angiotensin II is approximately 15- to 30-fold less potent than unconjugated angiotensin II in inducing ileal and gallbladder contraction. Furthermore, the hypertensive potency of angiotensin II is reduced by approximately 30-fold upon sulfonation (327).

C. Peptidyl sulfotransferases

To date, two tyrosyl-protein sulfotransferases capable of sulfonating the tyrosine residues of proteins and peptide hormones have been cloned and localized to the *trans*-Golgi network (106, 107, 208). Whether either of these enzymes is responsible for CCK and/or gastrin sulfonation or whether other specific sulfotransferases are involved is presently unclear. ProCCK, with a molecular mass of 12,826 kDa, contains three tyrosine residues that are subject to sulfonation. One of the tyrosines is retained in the final active peptide, whereas the other two tyrosines are present in the extended carboxy terminus of the prohormone that is eventually cleaved during processing. For neuropeptides that are commonly sorted as precursors and processed proteolytically in secretory granules, sulfonation usually precedes and, thus, can influence the proteolytic processing of these precursors. In a study (328) in which inhibition of protein sulfonation was employed, however, proteolytic processing of precursor proteins was unaffected, suggesting that protein sulfonation is not required for intracellular transport, sorting, and processing. In another study employing mutational analyses (104), it was suggested that tyrosine sulfonation alters the amount of CCK secretion but is not an absolute requirement for processing and secretion. This finding notwithstanding, sulfonation may still be important regarding solubility and stabilization (104).

D. Glycoprotein hormones

Sulfonation of *N*-acetylhexosamines on the α-subunit of LH was the first posttranslational modification of a pituitary hormone reported (180). Subsequently, a similar posttranslational modification of the β-subunit of LH was discovered (329). Furthermore, LHRH stimulates secretion of both sulfonated LH subunits (330). The structure of the sulfonated asparagine-linked oligosaccharides on human LH contains

the peripheral sequence SO_4-4N-acetylgalactosamineβ1, 4N-acetylglucosamineβ1,2Manoseα (SO_4-4GalNAcβ1, 4GlcNAcβ1,2 Manα; Refs. 331–333). Human TSH contains the same sulfated peripheral sequence and to a similar extent, whereas human FSH lacks this modification (283). The single glycosylation site on the human LH β-subunit contains the greatest proportion of disulfated oligosaccharides, whereas one of the two sites on the α-subunit contains the greatest proportion of monosulfated structures (283). In contrast to the pituitary hormones, HCG does not contain either sulfate or *N*-acetylgalactosamine on its asparagine-linked oligosaccharides (334). Sulfonation of the LH oligosaccharides does not alter bioactivity at the level of interaction with the LH receptor but does influence bioactivity by regulating its half-life in the circulation (335).

A receptor on hepatic endothelial and Kupffer cells that recognizes oligosaccharides terminating with the sequence SO_4-4GalNAcβ1,4GlcNAcβ1,2Manα has been identified as being responsible for the rapid removal of LH from the circulation (336). Importantly, the rapid removal of LH from the circulation in association with the accelerated release of LH from pituitary gonadotrophs accounts for the characteristic episodic rise and fall in levels of plasma LH, a feature that is considered essential for maximal bioactivity (10). A similar mechanism exists for TSH, *i.e.*, TSH contains the same SO_4-4GalNAcβ1,4GlcNAcβ1,2Manα sequence that can interact with the specific hepatic receptor, resulting in its rapid clearance from the circulation (337, 338). As a consequence of the rapid plasma clearance of sulfonated TSH, pulsatile variations in plasma TSH levels are generated (338–340). As in the case with LH, an episodic pattern of TSH secretion from the human pituitary in association with the rapid clearance of TSH from the circulation is considered essential for maximal bioactivity (341). Thus, LH and TSH have similar properties regarding half-life, and receptor activation that is dependent on asparagine-linked oligosaccharides containing sulfonated caps.

POMC is the protein precursor of several pituitary peptides and hormones, including ACTH, β-endorphin, and α-MSH (342). Processing of POMC into bioactive peptides involves several cleavage steps. In addition to proteolysis, several other posttranslational modifications are known to occur, including glycosylation (343). Furthermore, POMC has been shown to contain asparagine-linked oligosaccharides terminating with the sequence SO_4-4GalNAcβ1,4GlcNAcβ1, 2Manα (286, 344). Thus, the presence of the SO_4-4GalNAcβ1, 4GlcNAcβ1,2Manα sequence on glycosylated cleavage products of POMC can influence their clearance from the circulation by interacting with the specific hepatic receptor recognizing this structural feature (286).

A glycoprotein has been isolated from rat liver that has the properties expected of the receptor mediating the removal of LH and TSH from the circulation by recognizing the SO_4-4GalNAcβ1,4GlcNAcβ1,2Manα pattern (345). Furthermore, the location of the sulfate group in the 4-position is critical. Glycans containing a terminal GalNAcβ1, 4GlcNAcβ1,2Manα sequence with a sulfate in the 3-position instead of the 4-position have a significantly reduced affinity for the specific hepatic receptor (345) and a 12-fold difference in the rate of clearance from the circulation (346).

E. N-Acetylgalactosamine-4-O-sulfotransferase

In all species studied, the α-subunit of glycoprotein hormones contains two asparagine-linked oligosaccharides, whereas the β-subunit contains either one (TSH and LH) or two (FSH and HCG) asparagine-linked oligosaccharides (284, 347). Protein precursor oligosaccharide conjugates are added posttranslationally and are subjected to further processing in the Golgi complex before secretion (348). The sulfotransferase capable of sulfonating the 4-hydroxyl group of *N*-acetylgalactosamine of the terminal asparagine-linked oligosaccharide on LH was initially identified in pituitary membranes (349). Interestingly, estrogen modulates expression of *N*-acetylgalactosamine-4-*O*-sulfotransferase and *N*-acetylgalactosamine transferase, the enzymes regulating synthesis of the terminally sulfonated oligosaccharides on pituitary glycoproteins (350). The cDNA for *N*-acetylgalactosamine-4-*O*-sulfotransferase has been cloned and encodes for a 424-amino-acid transmembrane protein that is highly expressed in the pituitary and other regions of the brain and is active with substrates terminating with GalNAcβ1,4GlcNAcβ-R including LH (351, 352). The gene for human *N*-acetylgalactosamine-4-*O*-sulfotransferase is located on chromosome 19q13.1, covers approximately 88 kb of genomic DNA, and consists of 4 exons (351, 352). A second human *N*-acetylgalactosamine-4-*O*-sulfotransferase has been cloned that consists of 443 amino acids and is 46% identical with the first *N*-acetylgalactosamine-4-*O*-sulfotransferase cloned (353). The gene for the second human *N*-acetylgalactosamine-4-*O*-sulfotransferase localizes to chromosome 18q11.2 and consists of five exons (353). In contrast to the high expression of the gene for *N*-acetylgalactosamine-4-*O*-sulfotransferase-1 in the pituitary and brain, expression of the gene for *N*-acetylgalactosamine-4-*O*-sulfotransferase-2 is essentially absent in the brain. Instead, the latter isozyme is predominantly expressed in the trachea, heart, liver, and pancreas (353). Furthermore, the fact that the two isozymes have distinct properties lends further support to the theory that sulfonated oligosaccharides of glycoproteins have a critical biological role (353).

VIII. Sulfonation of Extracellular Structures and Signaling

A. General

Carbohydrates are fundamental to virtually every aspect of extracellular traffic, fulfilling roles from purely structural to mediating highly specific recognition events underlying extracellular signaling and cell-cell communication (5). Furthermore, extracellular sugars are commonly subject to covalent modifications that create additional structural variety, and among these modifications, one of the most prevalent is sulfonation (5). The highly charged sulfonate groups remain fully ionized at any pH encountered in a biological system providing an anionic component for electrostatic interactions. Importantly, the sulfonation of carbohydrates is now recognized as a mechanism for generating unique ligands for specific receptor-binding activity (9). The glycosaminoglycan heparan sulfate is an example of a structure exhibiting

extensive variation as a result of differential sulfonation, thereby presenting a diverse array of unique motifs with the potential for binding specific receptors. The broad utility of such adaptive scaffolding is seen by the great variety of cell-surface interactions that are mediated by specific heparan sulfate glycosaminoglycans, including the binding of growth factors and cytokines (5). Additionally, the sulfonation of terminal oligosaccharides intrinsic to glycoproteins generates unique ligands as for example with the pituitary glycoprotein hormones presented in *Section VII.* The biological significance of such diversified schemes of carbohydrate sulfonation is that they underlie a multitude of processes such as organ development, extracellular signaling, and adhesion of leukocytes to endothelial cells at sites of inflammation (354). Thus, it is evident that the sulfotransferase enzymes generating the large diversity of unique sulfonated carbohydrate structures are of crucial importance.

B. Heparan sulfate

The sulfonated glycosaminoglycans, heparin and heparan sulfate, are common components of proteoglycans, which are glycosaminoglycan chains covalently bound to a protein core and increasingly implicated in a variety of specific cellular processes. In contrast to heparin, which is confined to mast cells, heparan sulfate has a ubiquitous distribution on cell surfaces and in the extracellular matrix (4). Cell-surface heparan sulfate proteoglycans are crucial to the binding and metabolism of serum lipoproteins (355) and the binding and uptake of thyroglobulin by thyroid cells (356). Furthermore, heparan sulfate is required for fibroblast growth factor (FGF) signaling by serving as part of a ternary complex involving FGF, FGF receptors, and heparan sulfate proteoglycans (3, 357, 358). For example, in a step requiring heparan sulfate, specific FGF isoforms have been implicated in testosterone-induced transformation of S115 mouse mammary tumor cells (359). FGF signals via binding to a specific high-affinity FGF receptor with intracellular tyrosine kinase domains, which results in receptor dimerization, autophosphorylation, and initiation of an intracellular signaling cascade (360).

FGF belongs to a family of about 20 related polypeptides that display biological activity toward cells of mesenchymal, neuronal, and epithelial origin (361), in which they are involved in such processes as cell growth, organ development, and angiogenesis (362). Similar to most other growth factors, FGF signally activates a receptor tyrosine kinase initiating a phosphorylation cascade within the cell (363). Unlike most other growth factors, however, the signaling complex assembled at the cell surface includes heparan sulfate. The FGF receptor family consists of four known members with many isoforms (364), and heparan sulfate proteoglycans are central to signaling through FGF/FGF receptor complexes (6, 365). A direct interaction between heparan sulfate proteoglycans and FGF receptors was revealed by the identification of heparan sulfate binding sites on FGF and FGF receptor tyrosine kinase (366). This crucial feature, central to FGF signaling, is emphasized by the high affinity binding of heparan sulfate [estimated dissociation constant (K_d) values of 1–100 nM] by members of the FGF family (366).

Syndecans are a family of transmembrane heparan sulfate proteoglycans comprised of four members that act as integrators of extracellular signals (367). Syndecans, which bind a variety of extracellular ligands via their covalently attached heparan sulfate chains, are expressed in a highly regulated manner and are cell type and developmental stage specific (6, 365). The main function of syndecans is to modulate ligand-dependent activation of primary signaling receptors at the cell surface, where syndecan core proteins target heparan sulfate chains to the appropriate plasma membrane compartment (365). Syndecans, via their extracellular glycosaminoglycan chains, bind a multitude of growth factors and extracellular matrix molecules and, via their small cytoplasmic domain, they interact with the actin-based cytoskeleton and potential downstream signal transducers (6, 365). Virtually all cell types express at least one form of syndecan, and most express multiple forms. Syndecan-1 is usually the major syndecan form found in epithelial cells, syndecan-2 is abundant in fibroblasts, and syndecan-3 in neuronal cells, whereas syndecan-4 appears more widely expressed (6).

C. Sulfotransferases

Essentially all mammalian cells synthesize heparan sulfate, present in basement membranes, on cell surfaces, and in the extracellular matrix. The first step in heparan sulfate/heparin chain assembly is the formation of a nonsulfonated precursor polymer by alternating glucuronic acid and *N*-acetylglucosamine units at the nonreducing end of the growing chain (Fig. 10 and Refs. 4 and 368). This step is catalyzed by a bifunctional protein expressing both glucuronic acid and *N*-acetylglucosamine transferase activity (369). The microheterogeneity of heparan sulfate structures is mainly produced by the nonrandom introduction of *N*-, 2-*O*-, 6-*O*-, and 3-*O*-sulfate groups (Fig. 10). Heparan sulfate polymers are first modified by partial *N*-deacetylation/*N*-sulfonation of *N*-acetylglucosamine residues. Further modifications include C5-epimerization of glucuronic acid to iduronic acid and *O*-sulfonation at various positions such as C2 of iduronic acid and glucuronic acid and C3 and C6 of *N*-acetylglucosamine (4, 368). Heparin, which is exclusively synthesized and stored in connective tissue mast cells, is more extensively and evenly modified along the polymer than is heparan sulfate, which is produced with greater structural variety (368).

The enzymes responsible for the biosynthesis of glycosaminoglycans are located in the Golgi apparatus. A tetrasaccharide "linkage region" attached to a serine residue in the core protein provides the starting point for polysaccharide chain elongation (Fig. 10 and Ref. 4). Once formed, the linkage region serves as the acceptor for an *N*-acetylglucosamine unit (Fig. 10), a step that commits the process toward generation of a glucosamino chain (4). In heparan sulfate synthesis, *N*-deacetylation and *N*-sulfonation of *N*-acetylglucosamine residues of the precursor polymer are carried out by a single bifunctional enzyme, *N*-deacetylase/*N*-sulfotransferase (370). This enzyme removes the acetyl group from *N*-acetylglucosamine and replaces it with a sulfonate group. Importantly, these reactions are prerequisite to all other modifications, because enzymes associated with the subsequent reactions require *N*-sulfate groups for recognition (87).

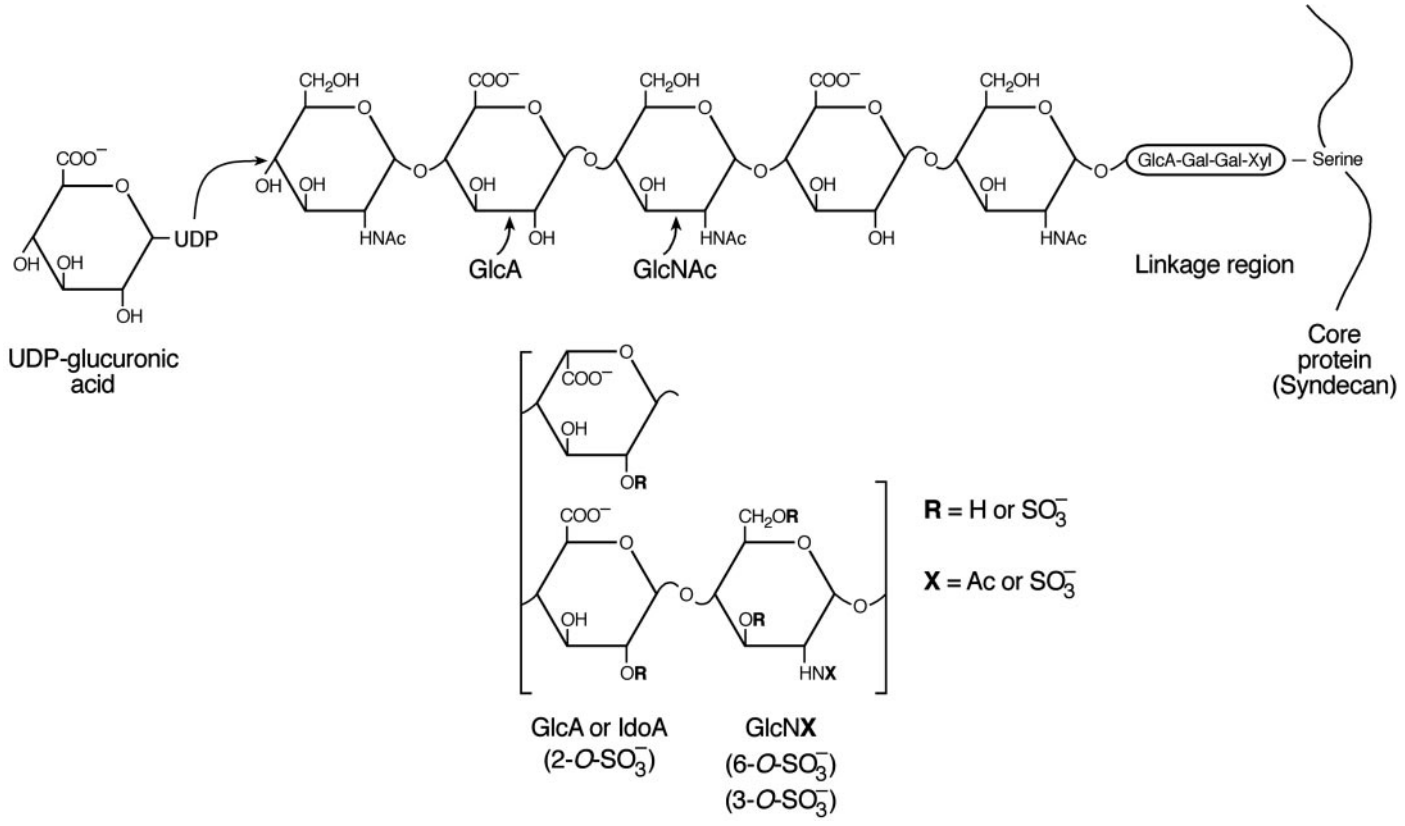

FIG. 10. Synthetic scheme of heparan sulfate. The growing glycosaminoglycan chain is covalently linked to a core protein (syndecan) at a serine residue by the encircled tetrasaccharide linker. Heparan sulfate is synthesized as an alternating polymer of glucuronic acid (GlcA) and *N*-acetylglucosamine (GlcNAc). Modifications occur as indicated *in the brackets*: GlcA can be inverted to form iduronic acid (IdoA) and either can be sulfonated at position 2; GlcNAc can be deacetylated and sulfonated at position 2 as well as sulfonated at positions 3 and 6.

Four isozymes of *N*-deacetylase/*N*-sulfotransferase have been cloned: *N*-deacetylase/*N*-sulfotransferase-1, located on chromosome 5q32–33.1 (371); *N*-deacetylase/*N*-sulfotransferase-2, located on chromosome 10q22 (372); *N*-deacetylase/*N*-sulfotransferase-3, located on chromosome 4q25–26 (373); and *N*-deacetylase/*N*-sulfotransferase-4, located on chromosome 4q26–27 (374). Disruption of *N*-deacetylase/*N*-sulfotransferase-1 in mice results in pulmonary hypoplasia and neonatal lethality (88, 375). Disruption of *N*-deacetylase/*N*-sulfotransferase-2 in mice indicates that this isozyme is primarily responsible for the production of heparin in mast cells (376, 377).

Heparan sulfate 2-*O*-sulfotransferase catalyzes sulfonation at position 2 of an iduronic acid residue in heparan sulfate and is essential for FGF2 binding and embryogenesis (5). Iduronic acid sulfate residues in different structural contexts promote interactions of glycosaminoglycans with a variety of proteins including FGF2, lipoprotein lipase, hepatocyte growth factor, and platelet-derived growth factor (378). In a gene trap screen designed to identify genes important in mouse embryogenesis, it was discovered that heparan sulfate 2-*O*-sulfotransferase is expressed differentially during embryogenesis, presumably reflecting changes in proteoglycan side-chain structure (379). Moreover, mice homozygous for the gene trap mutation exhibit bilateral renal agenesis, as well as defects in the eye and skeleton, emphasizing the importance of 2-*O*-sulfonation of heparan sulfate in embryonic development (379).

Glucosaminyl residues that are 3-*O*-sulfonated are rare constituents of heparan sulfate but are essential for antithrombin binding and clotting (5, 87). The human cDNA for 3-*O*-sulfotransferase has been cloned and expressed (380). Subsequently, four additional 3-*O*-sulfotransferases were cloned, expressed, and characterized (381). Generation of specific disaccharides containing 3-*O*-sulfonated glucosamines is dependent on the sugar structures around the targeted glucosamine residue and their recognition by specific isoforms of heparan sulfate 3-*O*-sulfotransferase, which are expressed at different levels in different human tissues (382). 3-*O*-Sulfotransferase-1 can be dramatically up-regulated by retinoic acid and cAMP treatment, and this occurs with little effect on the other sulfotransferases that modify heparan sulfate, suggesting a different transcriptional regulatory mechanism (383). Interestingly, there is evidence suggesting that a form of 3-*O*-sulfonated heparan sulfate may play a critical role in placental function (383).

Growth factors such as FGF2 and hepatic growth factor recognize a unique domain in heparan sulfate that includes a 6-*O*-sulfonate of glucosamine (5). Human heparan sulfate glucosaminyl-6-*O*-sulfotransferase has been cloned, expressed, and characterized, and no significant amino acid sequence identity to other proteins including other carbohydrate *O*-sulfotransferases was noted (384). Three isoforms of glucosaminyl-6-*O*-sulfotransferase with different specificities and expression patterns have been isolated from a

mouse brain library (385). These results suggest that the expression of the three glucosaminyl-6-*O*-sulfotransferase isoforms may be regulated in a tissue-specific manner; furthermore, each isoform may be involved in the synthesis of heparan sulfates with tissue-specific structures and functions.

IX. Summary and Future Directions

A principal objective of this review was to emphasize the extensive nature of the sulfonation process in biology, with particular emphasis on its involvement in endocrine systems. Similar to other forms of molecular modification, sulfonation is a vital procedure, and disruptions in the process can have significant pathophysiological ramifications and developmental consequences. The addition of sulfonate groups creates a negatively charged molecular environment at any physiological pH that can induce conformational changes, alter solubility, and promote ionic interactions, all of which can significantly influence biological activity. Furthermore, site-specific sulfonation of glycoconjugates generates unique epitopes that can be recognized by hormones, cell-surface receptors, and extracellular matrix proteins and viruses (386). Because sulfoconjugation reactions are potentially reversible, the biological effects of sulfonation are likewise potentially reversible (108). Thus, an on/off regulatory mechanism is created. Nonetheless, the continuing problem with sulfonation is that, despite its extensive occurrence involving essentially all classes of compounds in all organ systems, the raison d'être for a specific sulfonation reaction is not always clearly understood. This is especially so for endocrine systems and their cognate hormones such as catecholamines and steroids, not to mention other related small endogenous compounds, *e.g.*, vitamins C and D.

Genetic abnormalities involving Golgi complex carbohydrate sulfotransferases have been described that are associated with specific developmental defects involving craniofacial structures (371) and macular corneal dystrophy (387). In addition, gene targeting experiments involving selective carbohydrate sulfotransferases acting on macromolecular structures have revealed their crucial role in the normal development of organ systems such as the kidney (379) and lung (88) and the formation of normally functioning mast cells (377). Gene targeting of a specific Golgi sulfotransferase involved in the synthesis of heparan sulfate results in neonatal lethality (375). On the other hand, although polymorphisms involving cytosolic sulfotransferases associated with altered function are not rare (388, 389), these have not been determined to be clinically significant. In part, this may be because many of the cytosolic sulfotransferases exhibit overlapping substrate specificities. Disruption of the mouse estrogen sulfotransferase gene (*SULT1E1*) results in reproductive abnormalities involving both male and female animals (176). Males develop testicular abnormalities in later adulthood but retain fertility, albeit at a somewhat reduced rate, whereas females are infertile as soon as they achieve sexual maturity. The nature of these sex-specific problems, however, remains to be clarified. It is anticipated that in the near future there will be additional reports on the targeted disruption of other cytosolic, as well as membrane-associated, sulfotransferase genes. In the latter category, for instance, gene targeting of peptidyl sulfotransferases has not been reported as yet. Although the issue of redundancy, particularly with cytosolic enzymes, is a significant problem, this powerful experimental approach should be pursued to attain a fuller and more comprehensive understanding of the physiological and developmental significance of specific sulfotransferase genes. The problem of redundancy, in some cases, may require the use of multiple knockouts, *e.g.*, enzymes involved in the sulfonation of catecholamines and iodothyronines.

Although it is recognized that mutations involving the PAPS synthase 2 gene result in relatively benign dwarfing disorders involving both the mouse and human species (56, 57), mutations involving the PAPS synthase 1 gene have not been recorded. Of course, it is quite conceivable that loss of PAPS synthase 1 function would be embryologically lethal, given that it is the principal, if not sole, PAPS synthase isozyme expressed in the brain and bone marrow. Nevertheless, targeted disruption of the gene for PAPS synthase 1 should be carried out to confirm or deny this conclusion. At the present time, an understanding of the relationship between the PAPS synthase 1 and 2 genes is not appreciated. It is an enigma as to why there are two genes and little is known of their transcriptional regulation and differential expression during growth and development. The latter represents an important area for future research. Additionally, the recent observation that PAPS synthase 1 has a nuclear localization is an interesting, although poorly understood, finding and represents another important area for future investigations directed toward understanding this intriguing phenomenon.

Acknowledgments

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